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THE RACIAL AND SOCIAL INCIDENCE OF CANCER OF THE UTERUS.

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THE following paper serves to show the very inadequate state of our knowledge in a field where abundant clinical material is available for investigation. An attempt has been made to collect and summarize the very scattered literature and to save others the task of examining the actual papers. Some of the authors misrepresent their own data, misquote those of others, are defective in arithmetic and make the task of anyone who wishes to examine their conclusions difficult. Some authors, when giving data for the incidence of cancer other than uterine, do not say whether the figures refer to one or both sexes ; and in statistics based on admissions to a hospital they do not say whether this is a women's hospital or a general hospital, or where the hospital is situated, data which are not always easy to obtain in another country. One hopes that this presentation of the literature may lead to further investigation of questions, such as the social incidence of cancer of the cervix in this country, upon which more information should be available.

RACIAL INCIDENCE.

(A) *Upon Jewish Women.*

The earliest statements on this subject are not based on statistical material. Braithwaite (1901) says, " . . . cancer of the uterus was seldom or never met with amongst the numerous Jewesses attending the gynaecological out-patient department of the Leeds General Infirmary (only one case in 10 years). The experience of the London Hospital where there is a special Hebrew department, is the same (only one case in five years against 178 amongst Gentile women)."

London.

The number of Jews in London is unknown and no official British statistics give data for Jews separately. Sorsby (1931) utilized the records of Jewish burial societies. He says, " . . . the Jewish community possesses a practically complete register of its deaths. This comes about through the extreme rarity of a Jew, whatever his mode of life, being buried in a non-Jewish cemetery." He based his study of cancer in Jews upon 20,422 entries during 1910–1925 in the books of the Burial Society of the United Synagogues ; the reasons for this restriction of the field are given in full in his book. All records of deaths from cancer were checked by comparison with the corresponding death certificates at the General Register Office. In Table I a comparison is made with data for the whole of England and Wales.

TABLE I.—*Deaths from Cancer of Uterus.* (Sorsby.)

1911–1920.	Total deaths of women from cancer. A.	Deaths from cancer of uterus. B.	B per cent of A.
England and Wales . .	224,503	40,175	17·9
Jews of London	550	58	10·5

Munich.

Theilhaber (1910) gives the data shown in Table II on the authority of H. Kirschner, and states that cancer of the uterus makes up 25 to 35 per cent of cancers* in the general population of women, in contrast to the 7 per cent shown by these figures for Jewesses. The age distribution of these 7 cases was as follows : 30–40, 1 ; 40–50, 2 ; 50–60, 2 ; 60–70, 2.

The seven cases of uterine cancer in Jewesses which appear in Table II appear to be identical with those in Table III.

* This is a very high percentage, for which Theilhaber shows no detailed evidence. The Registrar-General's Statistical Review (1935–1939) gives the following figures for England and Wales for the five years 1935–1939 :

Deaths of women from all forms of malignant disease 175,366
Deaths from malignant disease of the uterus . . . . 22,303 or 12.7 per cent.

The corresponding figure given by Fischberg (Table XI) for a largely non-Jewish population, apparently of women only, is 16 per cent. See also the data from Amsterdam (Table VII ; 14 per cent), and from the Mayo Clinic (Table XVII ; 16·5 per cent). However, Theilhaber and Greischer (Table III) give a figure (28.7 per cent) from Munich in the later years (1907–09) of the period dealt with by Theilhaber which confirms his statement.

TABLE II.—*Deaths of Jews from Cancer, Munich*, 1876–1908. H. Kirschner, published by F. Theilhaber, 1910.)

	Males.	Females.
Deaths of Jews from cancer	87	98
Cancer of uterus	—	7
Cancer of stomach and intestines	—	41

Theilhaber and Greischer (1910) record the number of deaths from cancer in Munich (1) in the whole population of persons aged 25 and over in 3 years, 1907–1909 (A); (2) in the Jewish population in 19 years, 1880–1908 (B).

Figures from A are given under "Christians" in their Table XIII, though one is not told whether the "whole population" of A includes Jews; but the number of Jewish deaths in 3 years might not introduce any serious error. In the same table, figures from the three years (1907–1909) of A are placed under the heading "1880–1908" with those of B. In spite of these careless errors, the data seem to be of some value and are summarized in Table III, which shows that cancer of the uterus formed a much higher percentage (28·7) of all cancers in the general population than among Jews (6·8), while cancer of the breast showed a difference in the opposite sense but of smaller amount (9·0 and 16·7).

TABLE III.—*Cancer of the Uterus and Mamma. Munich* (Theilhaber and Greischer, 1910).

Christians (1907–1909).

Total cancers in women.	Uterus. —	Uterus. Per cent of total.	Mamma. —	Mamma. Per cent of total
1326	381	28·7	120	9·0

Jews (1880–1908).

Total cancers in women.	Uterus. —	Uterus. Per cent of total.	Mamma. —	Mamma. Per cent of total.
102	7	6·8	17	16·7

Population.	Jews.	Total.
1880	4,144	230,023
1905	10,056	538,983

The data (Table IV) from his own patients given by A. Theilhaber of Munich (1909) show that the ratio of cases of myoma to those of cancer of the cervix was, in the whole series of 5848 patients, 228 : 133 or 1·7 : 1, while among Jewesses the ratio was 43 : 1. These ratios do not, of course, exclude a high incidence of myoma upon Jewesses.

Vienna.

Peller (1931) examined the official statistics (no exact reference is given) of Vienna, where the Jewish population numbered 200,000, and gives data upon which Table V is based. He states that similar differences are shown in each

TABLE IV.—*Social Incidence of Carcinoma of Cervix.* (A. Theilhaber, 1909.)

Occupation of husband.	Private means.	Manufacturers, merchants, "Kommerzienraten."	Higher officials, medical practitioners, lawyers.	Lower officials.	Tradespeople.	Independent manual workers.	Inn-keepers, butchers.	Manual workers, employees.	Labourers.	Agricultural labourers.	Occupation unknown.	Total.	Jewesses.
Patients	80 = 35 per cent												
Myoma	19	49	12	27	6	23	5	15	49	7	16	228	43 = 19 per cent
Carcinoma of cervix	—	4	1	20	7	8	28	7	45	8	5	133	1 = 0·75 per cent
	5 = 3·7 per cent											361	
Per cent													
Myoma	8	22	5	12	2	10	2	7	22	3	7	100	
Carcinoma of cervix	—	3	1	15	5	6	21	5	34	6	4	100	

one of the four years in question. Cancers of the uterus made up three-quarters of the cancers of the female genital tract in non-Jewish women, but less than one-half of these tumours in Jews, while cancers of the ovary provided one-half of the Jewish genital cancers and only 18 per cent in other women. Jewesses provide 7·63 of all genital cases, 18·4 per cent of cancers of the ovary and only 4·95 per cent of cancers of the uterus.

Ovarian tumours tend to occur at an earlier age than does uterine cancer, but Peller says that there was no difference in age distribution between the two groups which would introduce this factor. He states that the menarche occurs much earlier in Jewish women than in "the whole female population," but gives no numerical data.

TABLE V.—*Deaths from Cancer of the Female Genital Tract. Vienna** 1926–1929. (Peller, 1931.)

	Vagina.		Uterus.		Ovary.		
	Deaths.	Per cent of total.	Deaths.	Per cent of total.	Deaths.	Per cent of total.	Total.
Jewish	3	2	76	48	79	50	158
Non-Jewish	106	6	1459	76	350	18	1915

* Davidsohn (1939) states that these data are from the Hadassah Hospital, Jerusalem, but Peller's table is headed quite clearly "In den Jahren 1926–1929 starben in Wien an Krebs."

Budapest.

Theilhaber (1910) gives figures, summarized in Table VI, and says that they are taken from the "*Statistische Jahrbücher der Stadt Budapest.*"

TABLE VI.—*Carcinoma of Uterus. Budapest* 1902–1906. (Theilhaber, 1910.)

	Total. A.	Jewish. B.	Jewish per cent of total.	Ratio B : A	Cancer of uterus per cent of all cancers.	
Deaths	71,700	12,605	17·6	1 : 5·7	Total	17·7
Deaths from cancer	4,426	883	19.9	1 : 5·0		
Deaths from cancer of uterus	783	68	8·7	1 : 11·5	Jewish	7·7

Presumably the total deaths, and deaths from cancer, are those of women only, but this is not stated. A rather larger fraction of the total deaths was due to all forms of cancer among Jews than among the general population, but Jewish deaths from cancer of the uterus showed a much lower ratio.

Sorsby (1931) also gives data for cancer of the uterus from Vienna and Budapest, which have been criticized by Peller (1931), and for cancer of the whole female reproductive tract from Warsaw.

Amsterdam.

Hoffman (1933) obtained data, some of which have been put together in Table VII, from manuscript material at the Municipal Bureau of Statistics in Amsterdam, for the incidence of cancer in relation to religious confession in that

city. The results are given under three headings, namely, Jews, Catholics and a third referred to as "Protestants" or "others." The figures show that the mortality from cancer of the uterus is much lower, and that from cancer of the ovary, and to a less extent that from cancer of the breast, is higher, in Jews. Unfortunately the absolute numbers of cases in the three groups are not given.

TABLE VII.—*Cancer Mortality of Religious Sects, Amsterdam*, 1920–1929. (Hoffman, 1933.)

	Percentage of all cancers in women. Cancer of—			Rates per 100,000 of both sexes. Cancer of—			Average age at death. Cancer of—		
	Ovaries.	Uterus.	Vagina.	Ovaries.	Uterus.	Mamma.	Ovaries.	Uterus.	
Jews	7·1	5·5	0·8	5·02	3·91	11·3	53·2	60·3	
Protestants	2·7	13·9	0·9	1·96	9·78	9·65	54·1	55·4	"all faiths."
Catholics	2·8	14·2	0·6	2·04	10·19	9·32			

Sorsby (1931) gives the following figures (Table VIII) taken from official publications to which no reference is given.

TABLE VIII.—*Cancer of Uterus. Amsterdam*, 1922–26. (Sorsby, 1931.)

	Number of deaths.	Rate per 100,000 women. All.	Aged 40 years and over.
Non-Jewish women	505	18·1	60·5
Jewish women	13	6·9	19·9

One must assume that these data are included in those given above from Hoffman for a longer period. The low incidence in Jews is the more noteworthy because they have, in Amsterdam, a somewhat higher proportion of older persons (Table IX).

TABLE IX.—*Amsterdam—Percentage of Persons of* 40 *Years of Age and Over.* (Sorsby, 1931.)

	1909.	1920.
Non-Jews	27·4	28·8
Jews	28·4	32·9

Sorsby (1931) quotes a thesis by J. Sanders which I have been unable to obtain, who, "speaking of Amsterdam, gives the incidence of cancer of the uterus per 100,000 individuals, 11·3 for Jewesses and 23 for non-Jewesses . . ."

Rotterdam.

J. Sanders (1916) gives data from Rotterdam (Table X) which show a low incidence of cancer of the uterus in Jews, and a still lower incidence upon members of two of the Protestant Churches (Christian Reformed and Reformed). He is unable to account for this difference among Protestants, which is shown also by figures for other forms of cancer and for tuberculosis, and cannot be explained by any difference in social conditions.

TABLE X.—*Deaths According to Religious Confession of Women in an Average Year of the Period* 1902–1914 *in Rotterdam per* 100,000 *Living.* (J. Sanders).

Cancer.	Dutch Reformed. W. Reformed.	Roman and Old Catholic.	Christian Reformed. Reformed Church.	Lutheran.	Jews.
Stomach	28·8	23·1	11·1	28·7	21·2
Uterus	23·8	22·8	5·2	21·1	8·2
Breast	9·4	10·4	4·8	15·3	14·7
All cancers	122·7	97·4	34·8	109·2	106·1

Sweden.

Dr. J. Heyman (1948, private communication) writes: "There are about seven thousand Jews in Sweden, i.e. about one per thousand of the population. The Radiumhemmet serves a well-defined area of the country which covers about half of the population. Since 1914 practically all cases of cancer of the cervix occurring within that area are referred to the Radiumhemmet for treatment. From 1914 to 1947 inclusive I have seen about 7000 cases of cervical cancer. Among those were three Jewish women. The "normal" figure would have been seven. Consequently the incidence is low. Our figures are, however, much too small for drawing conclusions."

The following publications, which I have been unable to see, are referred to by Davidsohn (1939). Auerbach (1908) "found the mortality rate in Budapest for uterine cancer per 100,000 of the population to be 8·6 for Jewish women and 25 for non-Jews." Adamowickowa (1929, 1932) published three papers in a Polish journal on the incidence of cancer on Jewish and other women in Warsaw, but, as quoted by Davidsohn, he does not distinguish cancers of the uterus from among all cancers of the female genital organs.

Palestine.

At the Copenhagen Cancer Statistics Conference in 1946, Dr. Karplus, Pathologist to the Municipal Hospital, Tel-Aviv, reported that cancer of the uterus was not common in Jewish women, but that the most striking thing was that the relation between the incidence of cancer of the body and cancer of the cervix was reversed in Jews. In his experience in Tel-Aviv cancer of the cervix was rare and there were four cases of cancer of the body to each one of the cervix. (At the Royal Cancer Hospital (Free), London, the ratio of cervix to body is about 6 or 7 to 1). He considered that this was almost certainly an hereditary factor and not likely to be due to special observances of the Jewish Law, the same facts being noted in unorthodox Jews.* Unfortunately Dr. Karplus gave no figures in support of this statement. Data showing an equally low incidence of cancer of the uterus, which is more difficult to demonstrate than a high incidence, in populations known to consist of orthodox, and of unorthodox Jews must have been difficult to obtain and would be of great interest.

* I am indebted to Prof. D. W. Smithers for this account of Dr. Karplus' contribution.

New York.

Fishberg (1902) gives data obtained (*a*) from the "Annual Reports" for 1898, 1899, 1900 of a Jewish hospital (Mt. Sinai) for both sexes; and (*b*) "from a general hospital which admits few Jews (St. Luke's)," both hospitals being in New York City (Table XI).

TABLE XI.—*Cancer of the Uterus at Mount Sinai and St. Luke's Hospitals, New York.* (Fishberg, 1902).

Parts affected by cancer.	Mount Sinai (Jewish), 9497 pts.			St. Luke's, 7933 pts.		
	Cancer cases.	Percentage of cancer cases.		Cancer cases.	Percentage of cancer cases.	
Uterus	18	7·0		59	16·0	
Breast	32	12·4		91	24·6	
Gastro-intestinal tract	81	31·3		48	13·0	
Liver	19	7·4	43·4	14	3·8	23·5
Rectum	12	4·7		25	6·7	
Other organs	38	14·7		86	23·2	
Sarcoma	58	22·5		47	12·7	
Total	258	100		370	100	

Table XI shows that cancer of the uterus, reckoned as a percentage of all cancers (Fishberg does not make clear whether the figures refer to females only) is less than one-half as common among Jewish women as among other races, while cancer of the breast shows a smaller difference in the same direction.

Smith (1941) studied the racial incidence of 3106 cases of cancer of the cervix observed at the Memorial Hospital, New York, from 1916 to 1937. He says:

"In an attempt to compare the nationality incidence in cervical cancer in this series with that of patients suffering from other gynecological lesions, consecutive records as filed have been studied, but the numbers used in each classification are not the entire number of patients filed under that diagnosis; i.e., this is a cross section of the clinic in the years studied rather than the total number of cases in the clinic."

I have been unable to learn from the Memorial Hospital the system upon which the cases making up this "cross section" were selected. Apparently a number of cases of other gynaecological conditions roughly equal in number (3062) to those of cancer of the cervix (3106) were taken. The results are summarized by Smith in Tables XII and XIII (his Tables IV and IV*a*).

The significance of these figures is affected by the very high proportion of benign tumours, hence in Table XIV and XIVA Smith's figures have been recalculated for the malignant tumours only.

In Table XIV the result of the whole investigation, which for some reason Smith does not give, namely, the ratio of cervix cancers to other conditions, is calculated from his figures. The results show that cancers of the cervix make up 58·5 per cent of all the conditions studied in the non-Jewish peoples and only 12·2 per cent in Jews, while if the non-malignant tumours are excluded the respective percentages are 82·3 and 48·4.

TABLE XII.—"*Comparison of Percentages of Nationality Incidence in the Several Types of Gynecologic Lesions.*" (Smith, 1941.)

	Cervix. %	Corpus. %	Ovary. %	Vulva. %	Benign. %	Clinic. No.	Clinic. %
Black	7·2	3·1	1·8	4·3	7·8	426	6·9
German	10·9	7·6	5·4	6·0	8·1	582	9·4
Italian	16·9	7·1	4·0	8·6	8·6	777	12·5
Jewish	4·2	19·6	21·6	7·8	35·5	1085	17·5
Greek	0·3	0·0	0·0	9·0	1·4	43	0.06
Irish	13·4	18·6	3·1	15·6	15·8	890	14·4
Polish	1·0	0·4	0·0	0·8	3·0	104	1·6
English and Scotch	10·6	0·0	4·9	0·0	8·6	439	7·1
Austrian	3·3	2·6	7·2	4·3	3·2	219	3·5
U.S.A.	24·5	35·2	40·7	50·4	5·6	1191	19·3
Others	6·7	5·6	5·4	1·7	1·0	412	6·6
Total	3106	445	221	115	2281	6168	—

TABLE XIII.—"*Comparison of Nationality Incidence in Patients with Cervical and Non-cervical Lesions and with a Cross Section of the Entire Clinic.*" (Smith, 1941).

	Cervix. No.	Cervix. %	Clinic. No.	Clinic. %	Non-cervical. No.	Non-cervical. %
Black	225	7·2	426	6·9	201	6·5
German	341	10·9	582	9·4	241	7·8
Italian	528	16·9	777	12·5	249	8·1
Jewish	132	4·2	1085	17·5	953	31·1
Greek	10	0·3	43	0·06	33	1·0
Irish	419	13·4	890	14·4	471	15·3
Polish	32	1·0	104	1·6	72	2·3
Scotch and English	330	10·6	439	7·1	109	3·5
Austrian	104	3·3	219	3·5	115	3·7
U.S.A.	764	24·5	1191	19·3	427	13·9
Others	221	7·1	412	6·6	191	6·2
Total	3106		6168		3062	

Vineberg (1919) gives data for the incidence of cancer of the cervix based upon material from 80,000 female patients at the Mount Sinai Dispensary and Hospital, New York. Table XV represents an attempt to reduce his rather confused statement to a clearer form. The higher proportion of cancer in the hospital cases as against those of the dispensary is attributed to better conditions for examination.

Vineberg (1919) points out that Jewesses of the poorer classes, to which most of those dealt with in his paper belong, are most likely to be orthodox in religious matters. "It is well known that the poorer classes of non-Jewish women not only do not observe such restrictions, but are in the habit of indulging in cohabitation during the menstrual period and very shortly after parturition."

TABLE XIV.—*Cases of Carcinoma of Cervix as Percentage of all Gynecological and of all Gynecological Malignant Cases. Memorial Hospital, New York,* 1916–1937. (Smith, 1941.)

	A. Carcinoma of cervix.	B. All malignant tumours.	C. All gyneco-logical cases.	A per cent of B	A per cent of C.
Black	225	248	426	90·7	52·8
German	341	395	582	86·3	58·6
Italian	528	579	777	91·2	68·0
Jewish	132	273	1085	48·4	12·2
Greek	10	10	43	100	23·3
Irish	419	527	890	79·5	47·1
Polish	32	35	104	91·4	30·8
English and Scotch	330	341	439	96·8	75·2
Austrian	104	137	219	75·9	47·5
U.S.A.	764	1069	1191	71·5	64·2
Others	221	260	412	85·0	53·6
				All	
Total	3106	3874	6168	79·9	50·4
				All non-Jewish	
Total non-Jewish	2974	3614	5083	82·3	58·5

TABLE XIVA.—*Cases of Carcinoma of Corpus as Percentage of all Gynecological and of all Gynecological Malignant Cases. Memorial Hospital, New York,* 1916–1937. (Smith, 1941.)

	A. Carcinoma of corpus.	B. All malignant tumours.	C. All gynaeco-logical cases.	A per cent of B.	A per cent of C.
Black	14	248	426	5·6	3·3
German	35	395	582	8·9	6·0
Italian	32	579	777	5·5	4·1
Jewish	85	273	1085	31·1	7·8
Greek	0	10	43	0·0	0·0
Irish	83	527	890	15·8	9·3
Polish	2	35	104	5·7	1·9
English and Scotch	0	341	439	0·0	0·0
Austrian	12	137	219	8·8	5·5
U.S.A.	157	1069	1191	14·7	13·2
Others	25	260	412	9·6	6·1
				All	
Total	445	3874	6168	11·5	7·2
				All non-Jewish	
Total non-Jewish	360	3601	5083	10·0	7·1

TABLE XV.—*Cancer of the Cervix in Jewish and Non-Jewish Women in New York.* (Vineberg, 1919.)

	Approximate proportion of Jewish patients.	New gynaecological patients.	Cases of "marked laceration of cervix."	Cancer of cervix. Jews.	Cancer of cervix. Non-Jews.
Mount Sinai Dispensary					
1893–1906	95%	19,800	1995	9 (1 in 2089)	9 (1 in 111)
1909–1918	14/15	*ca.* 30,000	—	13 (1 in 2154)	7 (1 in 285)
Mount Sinai Hospital		New female patients.			
1911–1918	14/15	*ca.* 30,000*	—	32 in 28,000 (1 in 875†)	33 in 2000 (1 in 61)

* Elsewhere in the same paragraph Vineberg says that these 30,000 cases included "carcinoma of the cervix 58 cases; of the uterus 35 cases . . . ," but gives no hint of the relation of these 88 cases to the 32 + 33 cases set down in the Table.

† Vineberg states this proportion to be 1 in 973.

Mayo Clinic.

Horwitz (1927) made a study of the records of all cases of primary carcinoma of the uterus (cervix or fundus) occurring in the Mayo Clinic between 1920 and 1925 inclusive (Table XVI). The proportion of Jews registering at the Mayo Clinic was estimated, by a method which is not very clearly described, at 5 per cent.

TABLE XVI.—*Analysis of* 1,237 *Cases of Primary Carcinoma of the Uterus from* 1920 *to* 1925 *inclusive at the Mayo Clinic.* (Horwitz, 1927.)

New patients registered at the Mayo Clinic (1920 to 1925 inclusive)	234,250
New Jewish patients (estimated at slightly less than 5 per cent)	11,700
Carcinoma of the uterus (Gentiles) (550 for each 100,000 Gentile registrations)	1,227
Carcinoma of the uterus (Jewesses) (86 for each 100,000 Jewish registrations)	10

	All women. Number.	All women. Per cent.	Jewesses. Number.	Jewesses. Per cent.
Married women	1202	97·0	10	100
Single women	35	3·0	0	0
Nulliparae	124	10·3	1	10
Total	1237		10	
Average number of pregnancies	4·43 in 1078 patients		4·67 in 9 patients	
Average age of women with carcinoma of uterus, years	49·93		45·40	

Horwitz also gives data from the Curie Hospital attached to the Mayo Clinic (Table XVII).

TABLE XVII.—*Analysis of Cases of Carcinoma at the Curie Hospital from* 1920 *to* 1925 *inclusive.* (Horwitz, 1927.)

	Gentiles.		Jews.	
	Number.	Per cent.	Number.	Per cent.
Registered from 1920 to 1925 inclusive	6630	—	206	—
Diagnosis of carcinoma	4402	66	129	62·0
		Per cent of carcinomas.		Per cent of carcinomas.
Carcinoma of the uterus	727	16·5	5	3·9
Carcinoma of the breast	1246	28·3	34	26·4
Carcinoma of the digestive tract, including liver and pancreas	753	17·1	39	30·2
Total	2726	61·9	78	60·5

Chicago.

Davidsohn (1939) studied the incidence of cancer of the cervix, and of the corpus, in the Mount Sinai Hospital, Chicago, during the years 1930–38 (Table XVIII).

TABLE XVIII.—*Carcinoma of Body and Cervix of Uterus, Mount Sinai Hospital, Chicago.* (Davidsohn, 1939.)

1930–1938.	Total.	Jewish.	Non-Jewish.
Female patients over 14	21,673	*ca.* 90%	*ca.* 10%
Endometria examined { Uteri, 942 / Curettings 1060 }	2002	—	—
Cervices examined { Uteri 942 / Cervices 136 }	1078	—	—
Carcinoma of body	21	15	6
Per cent of endometria*	—	0·83	3
Carcinoma of cervix	16	9	7
Per cent of cervices*	—	0·93	6·4

* These percentages, given by Davidsohn without explanation, will be found to imply that the ratio—90 Jewish to 10 non-Jewish—shown by the hospital population applies also to both parts of the uterus examined, which is, of course, not necessarily the case.

New York, Chicago and Philadelphia.

Weir and Little (1934) collected data from 18 general hospitals in Chicago, New York and Philadelphia showing the distribution of "cancer of the cervix and uterus" among Jewish and other women. They give a table, which is given in an abbreviated form in Table XIX, showing "Comparison of Jews among cases of cancer of the cervix and uterus with total admissions in eighteen general hospitals." Presumably the "total admissions" are those of women only.

TABLE XIX.—"*Comparison of Jews among Cases of Cancer of the Cervix and Uterus with Total Admissions in Eighteen General Hospitals.*" Weir and Little (1934).

Patients 1927–1931 inclusive.			Cancer cervix and uterus.		
Total.	Jewish.	Per cent Jewish.	Total.	Jewish.	Per cent Jewish.
572,232	111,447	19·48	1842	126	6·84

Weir and Little give another table of data from five Jewish hospitals in New York, Brooklyn, Cleveland and Philadelphia (Table XX).

TABLE XX.—"*Comparison of Jews among Cases of Cancer of the Breast and Cancer of the Cervix and Uterus in Five Jewish Hospitals.*" Weir and Little (1934).

Cancer of cervix and uterus.			Cancer of breast.		
Total cases.	Jewish cases.	Per cent Jewish.	Total cases.	Jewish cases.	Per cent Jewish
199	72	36·18	448	310	69·2

Unfortunately the authors speak only of "cancer of the cervix and uterus." I have been unable to ascertain whether figures for cancer of the cervix and of the corpus uteri separately are available.

Weir and Little (1934) give a graph (Fig. 1) showing the percentage age distribution in quinquennial periods of 598 cases of "uterine cancer" in Jewish and other women from eight hospitals in New York, Philadelphia, Cleveland and Chicago. The maximum is in the age group 50–54 in Jewish women and in that of 45–49 in others. The authors suggest the possibility that this difference depends upon racial factors. Certainly the age at which some forms of cancer occur is affected by genetic differences; examples have been given in detail in another paper (Kennaway and Kennaway, 1944). But there is nothing in these data for age distribution to indicate whether the liability to uterine cancer, which is the chief subject considered here, is affected by racial or by extrinsic factors. A larger proportion of cancers of the corpus uteri among Jewish women would have a similar effect (*cf.* Karplus p. 183; "Genetic Factors" and "Cancer of the Corpus Uteri," pp. 204, 205, below).

Summary of data on cancer of the uterus in Jewish women.

Table XXI brings together the data of different authors, which are expressed in a variety of ways, and cannot be referred to any one base-line; some are derived from the statistics of whole countries and cities, others from admissions to one or more hospitals.

The figures for cancer of the uterus, reckoned as a percentage of all cancers in women, range in the first seven cases in Table XXI from about 28 to 14 in non-Jews, and from 10 to 4 in Jews, the mean values being about 20 and 7. A similar ratio of the order of 3 : 1 is given by the death rates per 100,000 of Hoffman (Amsterdam), Sorsby (Amsterdam), Sanders (Rotterdam), Auerbach (Budapest); and by the figures of Davidsohn (Chicago) for cancer of the corpus uteri.

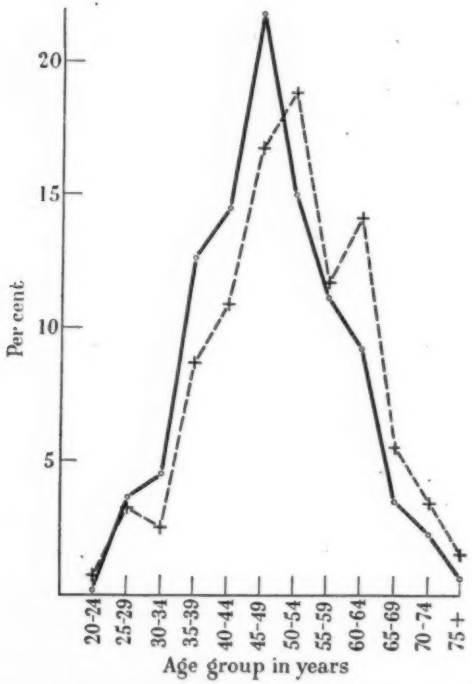

FIG. 1.—Age distribution of uterine cancer (Weir and Little, 1934). ——— Gentiles, - - - - Jews.

A smaller difference, of the order of 2 : 1 or less is shown by the data of Sanders (Amsterdam), Peller (Vienna), and by the hospital admissions for malignant tumours in women of Smith (New York). Other results from hospitals (Horwitz, Mayo Clinic; Davidsohn (cervix) and Vineberg) show much larger differences, up to 19 : 1. The difficulty of comparisons based upon admissions to hospitals is of course well known. Three items in Table XXI (F. Theilhaber, 1910; A. Theilhaber, 1909; Weir and Little, 1934) are not comparable with the rest. But all the 20 sets of data in Table XXI agree in attributing to Jewish women an incidence of cancer of the uterus lower than in other women, with the single exception of those of certain Dutch Reformed sects (Table X).

B. *Cancer of the Uterus in Hindu, Moslem, Parsee and Indian Christian Women.*

Statistics based upon attendance at hospitals are notoriously liable to error, more especially when they refer to people differing in race and religion. However, the following figures (Table XXII) should at any rate encourage further investigation. They are taken from the valuable collection of data on cancer in India obtained by Nath and Grewal (1935) from the records of in-patients of 13 hospitals in Punjab, Delhi, United Provinces, and Bihar and Orissa, comprising 6395 cases of cancer in all. The figures as they stand show a lower proportion

TABLE XXI.—*Cancer of the Uterus in Jewish Women. Summary.*

Author.	Locality.	Cancer of uterus.	Non-Jews.	Jews.
Theilhaber (1910)	Munich 1876–1908	Per cent of all cancers in females	25–35	7
Theilhaber & Greischer (1910)	Munich	Ditto	28·7 (1907–9)	6·8 (1880–1908)
Sorsby (1931)	London 1911–1920	Ditto England and Wales, whole population	17·9	—
		„ London	—	10·5
Theilhaber (1910)	Budapest 1902–1906	„ total population (? female)	17·7	6·7
Hoffman (1933)	Amsterdam 1920–1929	„ Protestants	13·9	5·5
		Catholics	14·2	—
Fishberg (1902)	New York 1898–1900	„ (? female) St. Luke's Hospital	16·0	—
		Mt. Sinai Hospital	—	7·0
Horwitz (1927)	Curie Hospital 1920–1925	„	16·5	3·9
Auerbach (1908)	Budapest 1901–1905	Death rate per 100,000 both sexes	25	8·6
Hoffman (1933)	Amsterdam	Ditto Protestants	9·78	3·91
		„ Catholics	10·19	
Sanders (1916)	Rotterdam 1902–1914	Death rate per 100,000 women : Reformed	23·8	8·2
		R. Catholic	22·8	
Sanders (1916)	Amsterdam	Ditto	23·0	11·3
Sorsby (1931)	Amsterdam 1922–1929	Death rate per 100,000 women	18·1	6·9
		Ditto aged 40 and over	60·5	19·9
Peller (1931)	Vienna 1926–1929	Per cent of all cancers of female genital tract	76	48
Horwitz (1927)	Mayo Clinic 1920–1925	Cases per 100,000 registrations (? sex)	550	86
Smith (1941)	New York 1916–1937	Per cent of all gynaecological cases	58·5	12·2
		Per cent of all malignant gynaecological tumours	82·3	48·4
Davidsohn (1939)	Chicago 1930–1938	Carcinoma of cervix per cent of cervices examined	6·4	0·93
		Carcinoma of body per cent of endometria examined	3·0	0·83
Vineberg (1919)	New York Mt. Sinai Dispensary	Ratio to admissions	1893–1906 1 in 111 (0·9 per cent)	1 in 2089 (0·048 per cent)
			1909–1918 1 in 285 (0·35 per cent)	1 in 2154 (0·046 per cent)
	Mt. Sinai Hospital	Ditto	1911–1918 1 in 61 (1·64 per cent)	1 in 875 (0·114 per cent)
Theilhaber (1910)	Budapest 1902–1906	Jewish per cent of all cancers (? females)	—	19·9
		Jewish per cent of all cancers of uterus	—	8·7
Theilhaber (1909)	Munich	Ratio uterine myoma : cancer of cervix	1·7 : 1 (all patients)	43 : 1
Weir and Little (1934)	New York, Chicago and Philadelphia	Per cent of admissions	—	19·5
		Per cent of "cancer of cervix and uterus"	—	6·8

TABLE XXII.—*Carcinoma in Hindu and Moslem Women.* (Nath and Grewal, 1935.)

	Cases of carcinoma in women.			Ratio A to C.
	A. Genital tract.	B. Breast.	C. All sites.	
Hindu	888	321	1830	1 : 2·06
Moslem	223	102	666	1 : 3·0

of uterine cancer in Moslem women, which might, of course, be due to various causes other than a true difference in incidence.

These results of Nath and Grewal are confirmed by the later and much more detailed observations of Khanolkar (personal communication) on the first 10,000 cases at the Tata Memorial Hospital, Bombay (Table XXIII), which, when calculated as percentages, show again an incidence of carcinoma of the cervix which is higher, in proportion to other forms of cancer, in Hindus (44 per cent) than in Moslem women (20·7 per cent). The Gujarati Hindus, who come from the coast to the north of Bombay, "form the bulk of the commercial community," while the Deccanis, from the south and east, "supply the industrial workers and the lower paid members of the clerical and teaching professions." Cancer of the cervix " . . . is most frequently met with in Deccani Hindu women. A badly balanced, deficient diet, during the child-bearing age may be a decisive factor in a population generally poorer than the rest." The figures in Table XXIII show a proportion of cervix cancer which is rather higher in the Deccani than in the Gujarati Hindus.

TABLE XXIII.—*Carcinoma in Women at the Tata Memorial Hospital, Bombay.* (Khanolkar.)

	Total female patients.	Total carcinomas in women.	Carcinoma of cervix.	Carcinoma of breast.	Per cent of total carcinomas in women.		
					Carcinoma of cervix.		Carcinoma of breast.
Hindus: Deccani	1180	699	329	98	47		14
Hindus: Gujarati	414	230	89	43	38·7	44·0	18·7
Hindus: Others	334	198	77	37	38·9		18·7
Moslems	495	275	57	65	20·7		23·6
Parsees	478	169	25	83	14·8		49
Indian Christians	543	224	69	61	12·7		27·2
Others	269	99	9	41	—		—
Totals	3713	1894	655	428			

In view of the possibility that Moslem women might avoid the Tata Memorial Hospital, which has a male medical staff, Khanolkar obtained data also from the Cama and Albless Hospital for Women, which is staffed entirely by women and is favoured by Moslem women. The figures (Table XXIV), reckoned on attendances and not on total carcinomas, as in Table XXIII, confirm those from the Tata Hospital.

TABLE XXIV.—*Carcinoma of the Cervix in Hindus and Moslems.* (Khanolkar.)

	Tata Memorial Hospital.			Cama and Albless Hospital.		
	Hindus.			Hindus.		
	Deccani.	Gujarati.	Moslems.	Deccani.	Gujarati.	Moslems.
Carcinoma of cervix per cent of total attendances	27·8	21·5	11·5	12·5	10·1	4·8
Ratio. Deccani = 1·0	1·0	0·78	0·41	1·0	0·81	0·39

The Indian Christians are descended from converts made by the Portuguese four centuries ago. "They are usually very devout Roman Catholic Christians and circumcision is not practised amongst them. There must have been a good deal of intermixture of Portuguese blood in the early days." Data from a Jewish population inhabiting the same area would be of great interest.

The Parsees show a percentage of uterine cancer (14·8) which is even lower than that seen in Moslems, and among the Indian Christians the percentage is lower still (12·7). Thus in India, the Hindu women on the one hand, and the Moslems, Parsees, and Christians on the other, appear, in so far as the very scanty data allow of any statement, to present a contrast similar to that between Gentiles and Jewesses in other parts of the world.

c. *Cancer of the Uterus in China.*

Maxwell (1929) in his book "*The Diseases of China*" says: "It is probable that uterine cancer ranks highest of all in the malignant growths in this country. Nor is this surprising considering how high the birth rate in the country districts is, or on the other hand, how frequent gonorrhoeal affection is in the cities and what a lack in both cases there is of cleanliness. In my own experience cancer of the cervix occurs in comparatively young people, it has often been seen in the thirties and in two cases in women between twenty and thirty. One of the most advanced cases of cancer of the cervix which I have seen was in a woman of twenty-three."

SOCIAL INCIDENCE.

The first to draw attention to the social distribution of cancer of the uterus appears to be A. Theilhaber, a gynaecologist of Munich, and his paper (1909) is perhaps the first to deal with the social, as distinct from the occupational, incidence of cancer on any one organ.

Bavaria.

Theilhaber (1909) records 133 cases of carcinoma of the cervix and 228 of myoma of the uterus among 5848 female patients treated by himself; cases of carcinoma of the body are excluded (Table IV). All the 361 cases are classified according to the occupation of the husband; nothing is said of the exclusion of any single women from the series. The numbers attending the practice of a single surgeon do not provide any basis for generalization and the age distribution among the various classes is unknown, but Table IV shows that the three highest social classes gave 35 per cent of the cases of fibroids and only 3·7 per cent of those of carcinoma of the cervix.

Theilhaber and Greischer (1910) give a classification of occupations in four groups, A to D, which is reproduced in Table XXV. Presumably Groups A to D referred to in other papers by A. Theilhaber (1909) and F. Theilhaber (1910) have the same significance.

TABLE XXV.—*Social Classes.* (Theilhaber and Greischer, 1910.)

Group A : Physicians, surgeons, lawyers, high officials, manufacturers, merchants, heads of firms, dentists, architects, persons of independent means.

Group B : Innkeepers, butchers, officials of middle rank, teachers, commercial professions not under A.

Group C : Independent manual workers, master craftsmen.

Group D : Domestic servants, journeymen, skilled craftsmen, bricklayers, mechanics, machinists, day labourers, unskilled workers, agricultural labourers, lower officials, pensioners, paupers, sempstresses, laundresses, factory hands.

Theilhaber and Greischer proceed to give the data shown in Table XXVI derived from the death certificates of women of ages over 25 in Munich for the years 1907–8–9. The proportion of deaths due to cancer of the uterus rises with descent in the social scale, while cancer of the mamma varies in the opposite direction, but in both series Group B shows an especially high incidence. There is no increase in passing from Group C (24·3) to Group D (24·6), but then the social difference between these two groups (Table XXV) is perhaps not very great.

TABLE XXVI.—*Deaths of Women, Munich*, 1907–8–9. (Theilhaber and Greischer, 1910.)

Social class of husband.	A. Total deaths.	B. Deaths from cancer.	Deaths from cancer of uterus. Total.	Per cent of A.	Per cent of B.	Deaths from cancer of mamma. Total.	Per cent. of A.	Per cent of B.
Group A	560	77	12	2·1	15·5	9	1·6	11·6
,, B	302	32	13	4·3	40·6	6	1·9	18·7
,, C	594	74	18	3	24·3	7	1·1	9·5
,, D	1373	239	69	5	24·6	19	1·3	7·9
Total	2829	422	112	3·96	26·5	41	1·45	9·7

F. Theilhaber (1910) followed the indications of the study by A. Theilhaber (1909) of his own cases by a wider investigation of the death certificates of Bavaria for 1908, which yielded 667 undoubted cases of uterine cancer. He proceeds to give a table (p. 480 of his paper) containing 863 cases, but does not comment upon the difference in these totals. The women are classified according to the husbands' occupation into four main classes, with 14 numbered and 35 named sub-divisions. In Table XXVII, only the four main classes are quoted.

Theilhaber does not state the populations of these various groups, and makes no comment upon this very serious deficiency. Figures for the age distribution of the populations of these classes were not available. The detailed table shows that, in 1908, in Bavaria apart from Munich, only a single case occurred among

TABLE XXVII.—*Deaths from Cancer of Uterus, Bavaria*, 1908. (F. Theilhaber.)

Social class of husband.	Deaths. Number.	Per cent of total.
Group A: Well-to-do-classes (wohlhabenden), independent, professional, commercial . .	62	7
„ B: Middle classes	60	7
„ C: Farmers, manual workers	114	13
„ D: Labourers	627	73
	863	100

the wives of ministers of religion, medical practitioners, lawyers, university teachers, engineers, merchants and manufacturers, while there was a high incidence upon the wives of inn-keepers and butchers.

Theilhaber then obtained the figures for deaths from cancer of the uterus from Munich only for the years 1906–1907, which were in Group A, 22, Group B, 22, Group C, 24, Group D, 135 (for Groups see Table XXV). In the absence of figures for the corresponding populations he took the numbers of deaths from all causes of women 24 years old (presumably he means " 24 years old and upwards ") for a few occupations only, for 1906 only, and by this not very laborious method obtained a result indicating an incidence of cancer of the uterus higher in Group B than in Group A. It seems impossible to reconcile some of his statements with some of his figures, but Table XXVIII is an attempt to reproduce his results.

TABLE XXVIII.—*Deaths from Cancer of Uterus, Munich*, 1906. (F. Theilhaber.)

Occupation of husband.	Number of deaths. Women—all causes.	Cancer of uterus.	Cancer of uterus per cent of all deaths.
Higher officials, university posts, artists, authors, merchants, manufacturers, shopkeepers, teachers . . .	273	3	1·1
Middle rank officials, butchers, inn-keepers	172	12	7·0

Theilhaber gives, finally, in a rather confusing form, data for the age at death from cancer of the uterus in the richer and poorer classes of Bavaria. He does not always make clear the occupations included in the two catergories of " Wohlhabende " and " Arme," but Fig. 2 and Tables XXIX and XXX have been prepared from the material, and these show the earlier age at death of the poorer women.

TABLE XXIX.—*Average Age at Death from Cancer of the Uterus, Bavaria*. 1908. (F. Theilhaber, 1910.)

Social class of husband.	Munich.	Five other large Bavarian towns.	Province of Unterfranken.
Group A: Well-to-do . . .	54·9	57·7	58·1
„ B: Middle class . . .	53·8	—	—
„ C: Manual workers . .	53·0	—	—
„ D: Labourers . . .	52·2	48·9	51·4

TABLE XXX.—*Age at Death from Cancer of Uterus in Bamberg, Augsburg, Würzburg, Erlangen, Nürnberg and the Province of Unterfranken* (1908) *and in Munich* (1906–7–8). (F. Theilhaber, 1910).

Age.	Wohlhabende.	Arme.
20–30	—	9
31–40	3	46
41–50	9	80
51–60	11	79
61–70	16	51
71–90	2	13
	41	278

Kauffmann (1926) in a lengthy study of 2000 cases at the Frauenklinik, Berlin, in the years 1912–1923, gives a table (p. 212) showing that 77 per cent of cervix cancers occurred in poor, and 23·3 per cent in rich, women, but he does not give the proportions of these two classes which attended the clinic.

England and Wales.

Data for the social distribution of cancer of the uterus (corpus and cervix), were given by the Registrar-General (1938) for the first time in the Decennial Supplement based on the census of 1931. This study was an expansion of that devoted to the social incidence of other forms of cancer in the preceding Decennial Supplement (1927) dealing with the .1921 census. In the publications of the Registrar-General (1927, 1938) " each census unit of occupation has been assigned to one of five graded Social Classes . . . after consultation with the Ministry of Labour. So far as is possible from the material available, Class I purports to represent the professional and generally well-to-do section of the population, Class III, skilled artisans and analogous workers, and Class V, labourers and other unskilled callings, while Classes II and IV are intermediate, comprising occupations of mixed types, or types not easily assignable to the classes on either side." Married women are classified according to the occupation of the husband as stated on the death certificate. " Only about 10 per cent of married women were recorded as gainfully occupied at the census of 1931, and of these about one third were employed in textiles and dressmaking, and about one third in domestic and personal service " (1938). Hence the mortality of the great majority of married women would show the effect of the environment to which they, and their husbands, are exposed, without that of any special occupational factors. (For a discussion of this matter see p. 3 *et seq.*, Registrar-General, 1938.)

The social class of single women is that indicated by their own occupation. The significance of the figures for married women has been discussed by Sutherland (1947).

The figures in Table XXXI are based upon 7831 cases of cancer of the uterus in married women, and 1294 cases in single women, in the years 1930–32. The table shows that the difference in mortality according to social class is considerable at every age both in married and in single women. Thus at ages 35–65 the rate in married women of Class V is twice that of Class I, and single women in Classes IV–V show a mortality 44 per cent greater than that of Classes I and II.

TABLE XXXI.—*Cancer of the Uterus. England and Wales*, 1930–1932.
(Registrar-General's *Statistical Review*, 1936, text, p. 90.)

Social class.	Mean annual death rate per million married women at ages:			Standardized mortality ratio (registered per cent of calculated deaths) at 35–65.		Per 1000 deaths from cancer of all sites at ages 65 and over.	
	35–	45–	55–65	Married.	Single.	Married.	Single.
Class I	119	264	469	65	88	78	62
,, II	144	348	515	78		96	
,, III	197	438	635	99	110	102	76
,, IV	239	466	627	106	127	110	95
,, V	294	591	754	130		110	
All	209	445	519	100		103	

The gradient of fertility shows a somewhat similar relation to social class. Thus in 1921 the ratio of registered to 100 calculated births for married males in the five social classes was 85, 85, 97, 109, 128 and the social distribution of uterine cancer in married women might be thought to be due to the difference in fertility. But the data for single women show that the matter is not so simple. "There must exist factors closely bound up with the social class differentiation, either selective or environmental or both, which are productive of uterine cancer quite apart from the parturient histories of the women concerned" (Registrar-General, 1938, p. 48).

United States.

Smith (1941) says that in New York City "Cancer of the cervix is found almost exclusively in the ward or clinic type of patient, rarely being seen in the type of patient who can afford private care."

France.

Dr. Denoix of the Ministère de la Santé Publique has been so kind as to inform me that no data are available of the professional and social incidence of cancer of the uterus in France.

FACTORS WHICH MAY AFFECT THE INCIDENCE OF CANCER OF THE UTERUS.

(1) *Ritual Observances and Ablutions Associated with Menstruation and Child-birth.*

(a) *Jewish ritual.*

The earliest Jewish law prohibits intercourse during menstruation or any other discharge of blood from the uterus, and for a period of seven days after the cessation of any abnormal flow. The general prohibition is expressed as follows:

Leviticus, XVIII, 19: "And thou shalt not approach unto a woman to uncover her nakedness as long as she is impure by her uncleanness."

Leviticus, XX, 18: "And if a man shall lie with a woman having her sickness, and shall uncover her nakedness; he hath made naked her fountain, and she hath uncovered the fountain of her blood; and both of them shall be cut off from among their people."

The precautions to be taken are stated in an earlier chapter, which makes plain the abhorrence, which is, of course, by no means restricted to Jews,* with which any contamination with genital blood was regarded.

Leviticus, XV, 19–28.—" 19 : And if a woman have an issue, and her issue in her flesh be blood, she shall be in her impurity seven days : and whosoever toucheth her shall be unclean until the even. 20 : And everything that she lieth upon in her impurity shall be unclean ; every thing also that she sitteth upon shall be unclean. 21 : And whosoever toucheth her bed shall wash his clothes, and bathe himself in water, and be unclean until the even. 22 : And whosoever toucheth any thing that she sitteth upon shall wash his clothes, and bathe himself in water, and be unclean until the even. 23 : And if it be on the bed, or on any thing whereon she sitteth, when he toucheth it, he shall be unclean until the even. 24 : And if any man lie with her, and her impurity be upon him, he shall be unclean seven days : and every bed whereon he lieth shall be unclean. 25 : And if a woman have an issue of her blood many days not in the time of her impurity, or if she have an issue beyond the time of her impurity : all the days of the issue of her uncleanness she shall be as in the days of her impurity : she is unclean. 26 : Every bed whereon she lieth all the days of her issue shall be unto her as the bed of her impurity : and every thing whereon she sitteth shall be unclean, as the uncleanness of her impurity. 27 : And whosoever toucheth those things shall be unclean, and shall wash his clothes, and bathe himself in water, and be unclean until the even. 28 : But if she be cleansed of her issue, then she shall number to herself seven days, and after that she shall be clean."

The law thus draws a distinction between normal menstruation (verses 19–24) and any other genital discharge of blood (verses 25–28). The normally menstruating woman ceases to be impure, if the flow has ceased, at the end of the seventh day from its commencement, but after any abnormal flow, a period of seven blood-free days is ordained, no doubt because there is greater danger of the recrudescence of any such bleeding. The law does not prescribe specifically for women any purification by washing such as is imposed upon men who have become contaminated. But subsequently, in Talmudic times† (about the third century A.D.), the law in regard to normal menstruation was altered in two respects, and these changes were set forth about 1000 years later in the Code Yoreh Deah : (1) The extension of the period of impurity until seven blood-free days had elapsed was applied to normal menstruation also. (2) The minimum first period, including the time of the actual flow, was laid down as five days. Hence the whole period of impurity is $n + 7$ days, and n is never less than 5, and under normal conditions will be 5. Such a period of 12 days is nearly one-half of the normal

* The supposed harmful influence of the menstruating woman upon men and children, and upon a great variety of objects (food and drink of every kind, cooking utensils, the domestic hearth, weapons, mirrors, crops, trees, livestock, and even upon roads and fisheries) is or has been the subject of stringent precautions and prohibitions among peoples all over the world, from Eskimos to Polynesians (for the immense literature on this subject see Ploss, Bartels and Bartels, 1927). Hence there is nothing in principle peculiar to the Jewish people in the ordinances of Leviticus, but they are characterized, as are other parts of the Mosaic law, by great precision in detail, and have received the voluminous addition of Talmudic literature.

† A tractate (Niddah) of the Talmud, consisting of 10 chapters, deals with this subject. An English translation of Niddah is available (Epstein, 1948). No translation of Yoreh Deah into a European language has been made ; the relevant portions of it (chapters 183–200) have been summarized by Sorsby (1931).

menstrual cycle and will allow intercourse to be resumed near what is thought to be the usual time of ovulation, of which process those who laid down the rule of course knew nothing.

The modern practice follows these rules. "The law of Niddah is in full force to the present day" (Epstein, 1948). The precautionary period of impurity begins 12 to 24 hours before the expected flow, and lasts for not less than 5 days from its commencement. The woman then examines herself and, if the flow has ceased, takes a bath. The second period, of seven days, during which she examines herself repeatedly, follows. If no blood has been found during this time, she takes another bath, followed by ritual immersion (Mikveh). The test for cessation of flow is made by inspection of a white cloth which has been pressed into the vulva.

Ritual immersion.

The Talmudists developed the Mosaic law of Purification by ordaining that a woman after menstruation should wash her body, and then immerse herself completely (two or three times) either in a lake, river, or spring, or in a tank containing not less than the equivalent of 24 cubic feet of water, which must either come immediately from the earth as a spring, or be collected rain water. In medieval times one of the first cares of every Jewish community was to provide a suitable bath for this immersion. In these buildings a staircase leads to a dressing-room and then on beneath the water to the floor of the bath, which was generally below ground-level so that the ground water, which could be regarded as spring water, could be utilized. Figures and architectural details of such baths are given by Ploss, Bartels and Bartels (1927, figs. 460–463) and in the Jewish Encyclopedia (1891) (article "Andernach").* A suitable bath can be constructed in a private house (Miller, 1930).

Mere immersion in spring water, as an addition to an ordinary bath, could make little contribution to cleanliness and was not intended to do so; hence, from the present point of view this particular ritual is in itself of little importance: but its persistence affords valuable evidence of an obedience to the law which probably would extend to other ordinances, and especially to the abstention from sexual intercourse. The object of the preliminary bath was not cleanliness *per se*, but the removal of anything which would intervene between the skin and the water during the total immersion, and thus deprive this ritual of its efficacy.† Some medical writers have emphasized, in rather vague terms, the importance of the cleanliness enforced by the Mosaic law, hence it is important to note exactly what the law requires. The washing, in a normal woman, at the beginning and end of the seven days, adds two baths a month to whatever other ablutions she may carry out.

* This account of Jewish ritual immersion is taken from various articles in the Jewish *Encyclopedia* (1891), from Ploss, Bartels and Bartels (1927) and from Preuss (1923). The Talmudic ordinances do not, of course, imply that women had not in earlier times purified themselves after menstruation by washing (see, for instance, the story of Bath-sheba, Sam. II, XI. 2–4).

† Chapter X of the tractate Niddah deals with numerous possible obstacles to the access of water to every part of the body. Thus the woman must not perform immersion in a harbour, because the passage of ships may stir up mud which would separate the skin from the water, and she must not raise her eyebrows unduly lest a wrinkle be produced into which the water does not penetrate. The ritual nature of the immersion is shown by the fact that, before it, all particles of food must be removed from between the teeth; although the mouth remains closed, there must be no potential obstacles to penetration. Such regulations are set forth in more accessible publications (*e.g.* Hurwitz, 1921) for use at the present day.

We have no data to show what proportion of Jewish women in any community carry out the whole procedure. One might form some estimate in London from the number of public ritual baths available, which are said to be not more than half a dozen; there are also some private ones. A new bath for this purpose was constructed in North London recently. But it seems probable that many women who do not go to the ritual bath may conform to the law in other respects, namely, the first and second bath, and abstention from intercourse, which are matters of purely private conduct.

The Mosaic law (Leviticus XII, 2, 5) relating to child-birth is important in view of the association of cancer of the cervix with parity. "If a woman conceive seed, and bear a man child, then she shall be unclean seven days; as in the days of the impurity of her sickness shall she be unclean. . . . And she shall continue in the blood of her purifying three and thirty days. . . . But if she bear a maid child, then shall she be unclean two weeks as in her impurity: and she shall continue in the blood of her impurity three score and six days." Thus the whole period of impurity was either $7 + 33 = 40$, or $14 + 66 = 80$ days. The practice in this country at the present time is said to be, to observe rather longer periods, of two and three months respectively.

(b) *Moslem law.*

The Koran (Sura 2) gives directions for the purification of women which are much less precise than those of the Jewish law. "They will ask thee also concerning the courses of women: Answer, they are a pollution: therefore separate yourselves from women in their courses, and go not near them, until they be cleansed. But when they are cleansed, go in unto them as God has commanded you." No periods of time are laid down, and no details of the method of washing are given. Obviously, immersion on the Jewish scale would be difficult in countries where water is scarce. Sale (1861) in his commentary on the Koran, speaks of two degrees of purification from various contaminations, namely, immersion or bathing in water, and washing of the face, hands and feet in water or (Sura 4 and 5) in sand if water is not available; and he goes on to say that the former is incumbent upon women after menstruation, but this is not in the text of the Koran.

(c) *Parsee ritual.*

The Zend-Avesta (Darmesteter, 1880) enacts that a woman having any normal or abnormal discharge of blood must be placed in a separate building [as is done in many parts of the world (footnote, p. 198)] and must be prevented as far as possible from defiling the elements of earth, water and fire; she must be given very scanty food lest the evil power (Ahriman) within her be strengthened, and the person who brings this food must not approach within three paces of her, and must use a metal spoon to convey the food. "If she still see blood after three nights have passed, she shall sit in the place of infirmity until four nights have passed," and so on up to a flow lasting 8 nights, one day being added to the actual period. Then "they shall dig three holes in the earth" (magas) "and they shall wash the woman with gomez" (urine of cattle) "by two of those holes and with water by the third." Intercourse with a woman having a normal or abnormal issue

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of blood is a crime punishable with 200 stripes. A flow of blood lasting more than nine nights is the work of evil spirits.

Dr. Modi (1922), a Parsee, quotes these laws and says: "At present also, most of the Parsee women generally observe the above practices. There are no separate Dastânistâns or houses for menses in Parsee towns or streets, but generally a sequestered part of one's own house is chosen for the purpose. The down-floor of the house was thought to be the proper place. But nowadays, in a crowded city like Bombay, the down-floor, instead of being a quiet and healthy place such as that contemplated by the early injunctions of the Vendidâd, is generally quite the contrary. So, most women in menses pass the period of menstruation on their upper floors, but in an isolated way. Every family has a separate iron cot for the occasion and a separate bedding, etc. They are supplied their meals from a distance by others and they neither come into contact with others, nor do they touch other things or do household work. The very rigorous isolation enjoined by the later books is not observed, but anyhow, some kind of isolation and separation is maintained by the generality of women. In the matter of taking food, very few use spoons now, though up to about 25 years ago, that was generally the case. In the matter of purification, they observe the bath enjoined by the early books, but the Vendidâd injunction of bathing over the three "magas" is not observed at all. A separate place of bathing and for purposes of nature for women in this condition is generally provided in Parsee houses."

(d) *Hindu ritual.*

Dr. Khanolkar (personal communication) says that the Parsee laws regarding menstruation are almost identical with those practised by orthodox Hindus and probably both of them are derived from early Aryan ritual. The Abbé Dubois (Beauchamp, 1906), writing about the beginning of the nineteenth century, quotes from the book *Padma-purama*, reputed to be the work of the hermit Vasishta, a rule for a 3-day period of isolation for the menstruating woman, followed by a day of ceremonies and ablutions, including 36 total immersions in a river. During the three days " . . . the mere wish to cohabit with her husband would be a serious sin." Elsewhere he says, "The mother of the newly-born child lives entirely apart for a whole month or more, during which time she may touch neither the vessels nor the furniture of the house, nor any clothes, and still less any person whatsoever. The time of her seclusion being over, she is immersed in a bath, or else a great quantity of water is poured over her head and body. Women are similarly isolated during the time of their periodical uncleanness. In all decent houses there is a sort of small gynaeceum set apart for them; but amongst the poor, in whose huts there is no such accommodation, the women are turned into the street, under a sort of shed or outhouse, or else they are allowed a corner of the cowshed. . . . When the time of uncleanness is passed, all the garments that the woman has worn are given to the washerwoman. Her clothes are not allowed inside the house; in fact, no one would even dare to look on them."

Jhaveri (1910) says that in all Hindu religious books minute directions for baths on very many occasions are given. During the first four days of the first menstrual period everything which the girl touches must be washed and " . . .

persons coming into contact with her have to take a bath." This author says nothing of subsequent periods. After childbirth a woman is impure for 10 days, even if given a daily bath by the midwife; " . . . no one dares to touch her."; after 40 days she is given a final bath, and is then pure (cf. the Jewish practice after the birth of a male child).

Ploss, Bartels and Bartels (1927) state, without giving any original authority, that in Malabar the first three days are spent in a special room of the house; on the fourth day the woman bathes and is then half-clean (i.e. can leave her room but not enter the temple), until the end of the seventh day. The three days of isolation, followed by ablutions on the fourth day, are obviously similar to the shortest procedure laid down for Parsees in the Zend-Avesta.

Dr. Khanolkar has very kindly sent me a translation from the Sanskrit of passages from the book *Dharmasindhu*, written about 1790 A.D. by Kashinath or Baba Padhye, which is the accepted basis of Hindu ritual and religious practices in the Bombay area. The menstrual woman must have no contact with other persons for three days and nights and is subject to numerous other prohibitions during this time. On the fourth day, after cleansing the body and after cleaning the mouth and teeth thoroughly, a full bath should be taken after sunrise at about the time when the cows are released to go to the pastures. This bath confers purification only for the purpose of touching ordinary household objects without polluting them, and for attending upon the husband. It is only on the fifth day that the woman is fit to participate in the worship of the gods and the ancestors. Dr. Khanolkar says that these regulations " . . . are still observed in the Hindu society, except by modern women in big towns, who work in schools, colleges, offices, etc., and cannot afford to remain in isolation every month."

The lenient Hindu view of intermenstrual bleeding is of interest in comparison with the Jewish law (p. 198). " If as a result of disease menses continue to appear continuously, the women is not impure and remains as if she is not menstruating. But she is debarred from participating in any acts of worship of the gods or the ancestors, and despite continuous or infrequent discharges she shall carefully work out a monthly period and remain under the enjoined discipline for three nights and three days once every month."

2. *Economic Conditions.*

The importance of these factors is indicated by the data collected under " Social Incidence " above, and especially by the large-scale statistics of the Registrar-General for England and Wales. The reference by Vineberg (1919) to the conditions under which the majority of his hospital patients in New York lived is valuable in this connection. " When one stops to consider that of the total number of the Jewish women 1995 had badly lacerated cervices . . . and that they were living in the worst possible hygienic surroundings, amidst the greatest squalor and privation, such as obtain in the lower East Side of the Metropolis, it is truly remarkable that so few cases of cancer of the cervix were detected amongst them."

3. *Child-bearing.*

The association of cancer of the cervix with parity (Lane-Claypon, 1927) makes the low incidence upon Jewesses all the more remarkable. (For references

to literature on the incidence in nulliparous women see Donaldson (1946) and Smith (1931)). Sorsby (1931) says, "Even a superficial acquaintance with the Jewish masses dispels the idea that Jewish women contain a large proportion of unmarried. The reverse is true; the number of unmarried is strikingly low—decidedly lower than among their neighbours. Nor do married women abstain from child-bearing. Procreation is almost a matter of religious observance with the mass of Jewesses. . . . Uterine cancer ought to be, if anything, more common among Jewish women than among non-Jewish."

Various Talmudic writers (Epstein, 1936, 29*b*–30*a*) laid down that a father should cause his sons to marry at 16 to 24, or 18 to 24, and that a daughter should "be dowered, clothed and adorned, that men should eagerly desire her"; no age is stated at which a woman should marry. The general tendency was to give daughters rather early in marriage; this led to the Rabbinic ruling that a man must wait for his daughter to grow up before giving her in betrothal (Epstein, 1936, p. 205). A modern compiler (Abramowitz, 1900) states the law of Israel on this matter thus: "After a man has arrived at the age of 18 it is his duty to take unto himself a wife in order that he may be fruitful and multiply, at any rate he should not pass his 20th year without having taken a wife. Having begotten a son and a daughter who are also generative, one has fulfilled the commandment "to be fruitful and multiply." . . . The command "to be fruitful and multiply" is not obligatory upon women; nevertheless a woman should not remain single lest she be liable to suspicion. . . . It is one of the mandates of the Sages that a man shall give his sons and daughters in marriage immediately they approach maturity. . . ." These Jewish practices thus laid down in the Law, and described by Sorsby as prevailing at the present day, should encourage early marriage.

The Abbé Dubois (Beauchamp, 1906) says that " . . . A Hindu only marries to have children, and the more he has the richer and happier he feels. . . . No Hindu would ever dream of complaining that his family was too large, however poor he may be . . . barrenness in a wife is the most terrible curse that can possibly fall on a family."

4. *Early Marriage.*

Lombard and Potter (personal communication) collected data from 549 cases of cancer of the cervix, and 550 cases of cancer of the breast, in State cancer hospitals of Massachusetts, and out of about 80 variables studied found 16 to be significant, and of these, marriage before the age of 20 was the most strongly associated with cancer of the cervix (Table XXXII). Thus 45 per cent of women with cancer of the cervix, and only 16 per cent of those with cancer of the breast, were married before that age. "Variables which might be considered are: Earlier childbirth, multiple children, poor obstetrical service, longer duration of married life, syphilis, immaturity of tissues at time of marriage, and excessive hormonal stimulation." Of these factors, longer married life could be eliminated by comparison with other groups. "One may surmise that a part, if not all, of the correlation between marriage under 20 and cancer of the cervix, which persists after elimination of the effect of the variables pertaining to pregnancy, economics, and syphilis may be due to either immaturity of tissue or to an oversupply of hormones. The latter may stimulate the individual to early marriage, and may also cause malignancy."

TABLE XXXII.—*Cancer of the Cervix and Marital Status. Massachusetts.* (Lombard and Potter.)

	Number in series.	Married per cent.	Without children per cent of married.	Married under age of 20, per cent of married.
Cancer of cervix: 2 series	549	96·0–97·7	9·3–10·8	44·0–49·2
„ uterus: 3 series	848	93·3–94·0	11·3–16·4	33·0–35·4
„ breast: 5 series	1509	81·5–84·4	16·1–21·7	11·3–22·9
„ other female organs: 1 series	234	83·3	20·5	21·0
First marriages of Massachusetts females, 1890–1939				18·6
Total female population; age-group 35–74.				
1940 census	—	83·2	17·2	—
1910 census	—	83·1	15·6	—

Modi (1922) writing of Parsees says that "the marriageable age at present is generally after 21 for the males and after 16 for the females." "The average age of Hindu women at marriage is 16 years, and of Parsee women 25 years." (Khanolkar).

5. *Genetic Factors.*

This factor is obviously a possible one, but its action is difficult to prove or disprove. A similar problem arises in the case of primary cancer of the liver in the African negro (Berman, 1941; Kennaway, 1944). Data are required from other Semitic peoples most nearly related to the Jews.* Weir and Little (1934) consider that the maximum incidence of uterine cancer one quinquennium later in Jewesses (Fig. 1) compared with other women, suggests a genetic basis. Certainly the age at which some forms of cancer occur is affected by genetic differences; examples have been given in detail in another paper [Kennaway and Kennaway (1944); xeroderma pigmentosum; familial polyposis intestini; cancer of the breast, gastro-intestinal tract and corpus uteri in certain families]. But a higher ratio of corpus to cervix cancer in Jewesses would have the same effect (Fig. 3).

6. *Circumcision.*

Some authors (Handley, 1936, 1947) consider that no explanation of the lower incidence of cervical cancer upon Jewesses need be sought beyond the (assumed) difference in the bacterial flora of the female genital tract brought about by this factor, a matter which it would be possible to investigate. More data from Moslem populations who practise circumcision, and from Parsees, who do not, are obviously very desirable. Handley (1936) has emphasized the value of statistics from Fiji, where the natives practise circumcision, in contrast to the Hindu immigrants, in whose native country phimosis and cancer of the penis are very common (Kennaway, 1947); certainly if these two races could receive equal medical attention interesting results might be obtained.†

Circumcision might act in another way, not dependent upon bacterial action. Phimosis, cancer of the penis and cancer of the cervix appear to be common in

* See "The Racial Origins of Jewish Types," by Radcliffe N. Salaman, in "Wherein I Glory," (London, 1948).

† The population of Fiji in 1945 included 115,724 Fijians and 117,256 Indians (*Statesman's Yearbook*, 1947). Brewster (1922) states that the majority of Indians were Hindus, " . . . although there was a fair proportion of Mohammedans."

certain areas (China, among Hindus in India ; Kennaway, 1947). If it be that material which accumulates under the prepuce contains carcinogenic compounds which can cause cancer of the penis, this material might, if conveyed in sufficient amount, cause cancer of the cervix. Plaut and Kohn-Speyer (1947) have shown that smegma (horse), and its unsaponifiable fraction, are carcinogenic to mice. But such transmission does not seem very likely.

7. *Douching.*

Comparative data for the incidence of cancer of the cervix in countries where douching is more or less commonly performed would be of great interest. Dr. Denoix of the Ministère de la Santé Publique has been kind enough to inform me that no data are available from France for comparison with those from this country.

Smith (1931) has described a positive correlation, of which there might of course be various explanations, between douching, and especially douching with lysol, and carcinoma of the cervix.

Cancer of the Corpus Uteri.

Cancer may affect either the cervix or corpus uteri, and in mass statistics, such as those based on death certificates, these two forms cannot be distinguished. This is very unfortunate because it is probable that they differ in etiology. We have no data which give directly the respective social incidence of cancer of the cervix, and of the corpus, but information should be available from hospitals which have separate accommodation for paying and other patients. The maximum incidence of cancer of the corpus falls about 10 years later than does that of cancer of the cervix (Fig. 3 and Table XXXIII), and this difference may suggest, when two batches of undifferentiated uterine cancers are compared, differences in the proportions of the two forms. For example : (1) The data from various parts of Bavaria given by Theilhaber (1910) shown in Tables XXIX and XXX show that the average age at death from cancer of the uterus is earlier in the poorer classes, and if these figures, admittedly very few in number, are put in graphic form (Fig. 2) they show a likeness to those for the two forms of cancer (Fig. 3), which is compatible with a higher proportion of cervix cancers in the poorer women. (2) If one reckons the death rates of married women in England and Wales of the four lower social classes from cancer of the uterus (Table XXXI ; *Statistical Review*, 1936, Text, p. 90) as percentages of the rate for Class I taken as 100, one obtains the result shown on the right-hand side of Fig. 4. The social difference becomes less as age advances from 35–45 to 55–65, which change is compatible with a decreasing proportion of cervical cancers, and suggests that it is this form which is affected by social factors.

The only authorities quoted in this paper who give any data for the incidence of cancer of the corpus upon Jewish and non-Jewish women are Smith (1941) and Davidsohn (1939). Smith's data (Table XIVA) show that cancers of the corpus make up practically the same percentage of all gynaecological conditions (7·1 and 7·8) in Jews and others, while the percentage of malignant gynaecological tumours made up by cancers of the corpus is much higher (31·1) in Jews than in others (10·0), owing to the smaller incidence of cancer of the cervix in Jews.

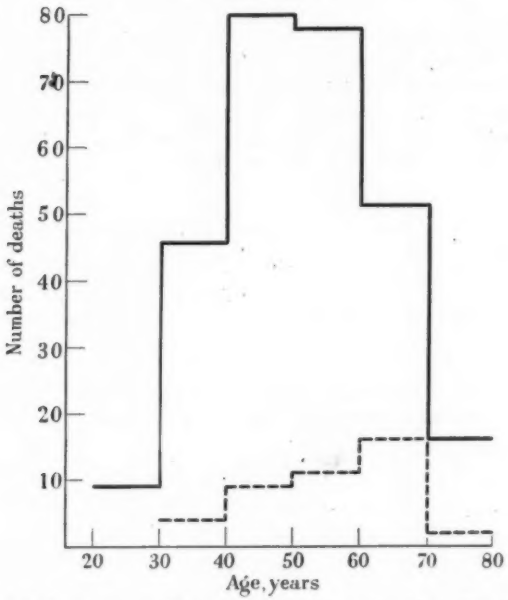

FIG. 2.—Age at death from cancer of uterus in Bamberg, Augsburg, Würzburg, Erlangen, Nürnberg and the Province of Unterfranken, 1908, and in Munich, 1906–7–8 (Theilhaber, 1910). Wohlhabende – – – – –, Arme ———.

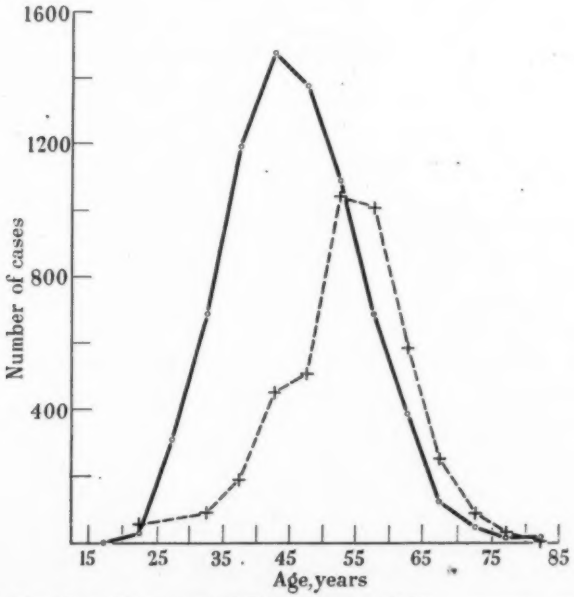

FIG. 3.—Cancer of cervix and corpus uteri (Lane-Claypon, 1927). . ——— . cervix, x – – – – x corpus (× 10)

Davidsohn's figures (Table XVIII) show much less difference between the percentages of specimens examined, made up by cancer of the corpus, and of the cervix, in Jewesses requiring gynaecological treatment (0·83 and 0·93) than in

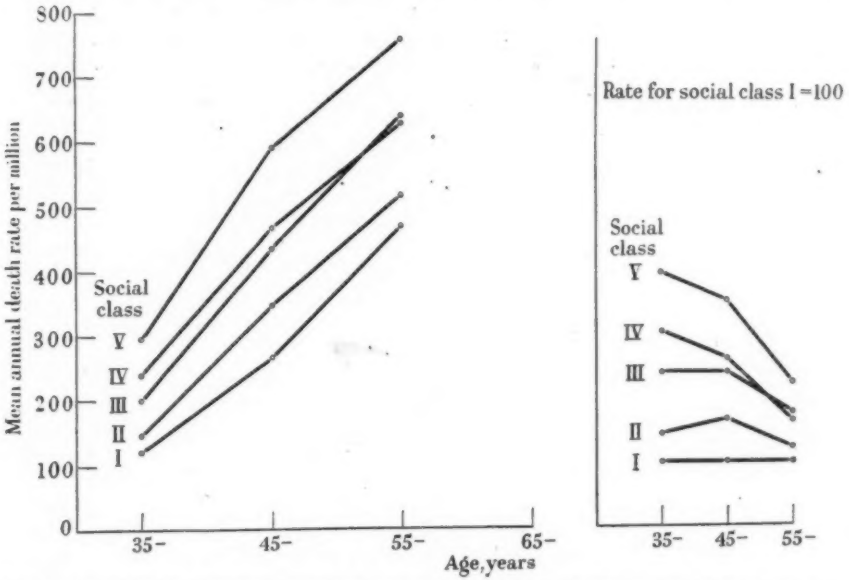

FIG. 4.—Social incidence of cancer of uterus at early and late ages. Mean annual death rate per million. Married women, England and Wales, 1930–1932.

other women (3·0 and 6·4). This difference is compatible with the idea that cancer of the cervix is the form of uterine cancer which occurs in Jewesses less than in other women.

DISCUSSION.

1. Any comparison of the figures for Jews and non-Jews (Table XXI) with those from the various communities in Bombay (Table XXIII) is possible only

TABLE XXXIII.—*Relation of Civil State and Age to Cancer of Cervix and Corpus Uteri in Various Countries.* (Lane-Claypon, 1927.)

	Cancer.	
	Cervix.	Corpus.
Total cases from literature . .	7986	389
Of these, unmarried . . .	170	52
	= 2·1%	= 13·4%
Mean age, years	45·75	53·3

	15—	20—	25—	30—	35—	40—	45—	50—	55—	60—	65—	70—	75—	80—
Cervix . . .	3	33	269	667	1196	1474	1370	1084	691	395	134	49	13	3
	Under 30.													
Corpus . . .	5		9		19	45	50	104	101	58	25	9	2	0

Totals: Cervix, 7381; Corpus 427.

on the basis of cancers of the uterus, or of the cervix, reckoned as a percentage of all cancers in women. Obviously these data were obtained under varying conditions, but such as they are, they are summarized in Table XXXIV.

TABLE XXXIV.—*Racial Incidence of Cancer of the Uterus.*

		Percentage of all cancers in women. Cancer of uterus.	
		Minimum.	Maximum.
Europe and U.S.A. (Table XXI)	Non-Jews	13·9	25–35
	Jews	3·9	10·5

		Cancer of cervix. Mean.
Bombay (Table XXIII)	Hindus	44
	Moslems	20·7
	Parsees	14·8
	Indian Christians	12·7

2. The Jewish ritual has some unique features. More or less complete isolation of menstruating women is practised by many other peoples and is laid down in the religious literature of some of these (Koran, Zend-Avesta, Dharmasindhu). Some quite primitive peoples (Aleuts; natives of the Congo and of Australia; for references see Ploss, Bartels and Bartels (1927)) are stated to enforce isolation at the menstrual period for as long as 6 to 7 days. But the Jewish ritual appears to be the only one which imposes an exact test for the cessation, and possible recurrence, of the flow, and a post-menstrual isolation of 7 days. The 5 + 7 day rule of the Jews is of peculiar interest in regard to the time of ovulation.

The Jewish rule suggests the following calculation. Suppose that a Jewish woman is married at 17, and undergoes the menopause at 47, and that during 5 of these 30 years the menstrual cycle is affected by pregnancy and its sequels. This leaves 25 years of normal cycles; if the average duration of these is 28 days, there will be $\frac{9125}{28} = 326$ periods, and $326 \times 7 = 2282$ days of abstention from intercourse after menstruation.

3. The low incidence of cancer of the cervix among Jews is the more remarkable because they tend to be exposed to factors (early marriage; child-bearing; in some areas low economic status) which in other communities predispose to this form of cancer; by some means they are able in this respect to resemble the richer women of other races.

4. The scantiness of the data available for Hindu, Moselm, Parsee and Indian Christian women does not allow one to draw any conclusions. In the Hindu community post-menstrual isolation of one day is combined with, in the male population, absence of circumcision and frequency of phimosis and cancer of the penis. The Parsees are of peculiar interest, as a one-day post-menstrual period is combined with absence of circumcision, but the only available data are those of the 25 cases of cervical cancer recorded by Khanolkar. These few cases are a very uncertain basis for any conclusions, but the much higher incidence upon Hindu women, who follow similar rules in regard to menstruation, suggest that these particular practices do not affect the matter, perhaps owing to the inade-

quate period of post-menstrual isolation. The four-day period of abstention possibly does not differ much from the practice of many persons whose action in this matter is not affected by religion. We seem to have no information about the actual procedures of isolation and purification among Moslems. The vast area and population of China afford only a few indications that cancer of the cervix, and of the penis, and phimosis, are all prevalent.

5. The social incidence of cancer of the cervix is obviously of great interest, but seems not to arouse attention among gynaecologists in this country. The social incidence upon married and single women illustrates the fundamental importance of Stevenson's work (Registrar-General's Decennial Supplements, 1927, 1938) to students of human cancer. Perhaps the cervix-corpus junction resembles that between the stomach and intestine in being the limit of influence of external factors.

The term "Single Woman" is a definition of civil state which may include more than one physiological condition. But there can be no doubt that the social gradation shown by the 1294 cases of cancer of the uterus in single women (Table XXXI) must depend upon factors less closely connected with sexual functions than are those discussed above under Jewish and other rituals. Possibly nutritional conditions are concerned here. These unique data are nearly 20 years old. After the next census it may be possible to learn whether any change has occurred which might be attributed to economic and dietetic differences.

6. One might make the following suggestions for further investigations :

(*a*) An inquiry into the incidence of cancer of the cervix in Jewish women who observe the 12-day period, the ritual immersion being disregarded. A beginning might be made by collecting the personal histories in individual cases.

(*b*) A comprehensive study by Indian workers of the unique material which they have at hand in Hindu, Moslem, Parsee and Christian populations.

(*c*) Further statistical study of the social gradation of uterine cancer in married and single women.

In all such investigations discrimination between cancers of the cervix and of the corpus should be attempted.

SUMMARY.

1. The comparative incidence of cancer of the uterus in Jewish and non-Jewish women has been studied in material from London, Munich, Amsterdam, Rotterdam, Vienna, Budapest, Sweden, Palestine, New York, Chicago, Rochester, and Philadelphia.

2. All of the twenty collections of data which have been found in the literature show an incidence of uterine cancer which is greater on non-Jewish than on Jewish women. These data are calculated upon several different bases and hence are not all comparable. Seven authors express the figures for cancer of the uterus as a percentage of all cancers in women, which percentage ranges from 28 to 14 in non-Jews, and from 10 to 4 in Jews, the mean values being 20 and 7. A similar ratio of the order of 3 : 1 is given by death rates per 100,000 in all of five instances. Data based on admissions to hospitals show, as would be expected, much wider variations.

3. The low incidence of cancer of the uterus in Jews is the more remarkable in that they are subject to some conditions (early marriage and child-bearing ; in

some communities low economic status) which in other peoples appear to increase the liability to this form of cancer.

4. Various degrees of isolation of the menstruating woman are, or have been, practised over a large part of the world. The Jewish ritual appears to be the only one which imposes an exact test for the cessation of the flow after 5 days, and for its possible recurrence during the following 7 days. This 12-day period of abstention from intercourse is of interest in regard to what is now known of the usual time of ovulation. The Jewish ritual immersion is probably of no direct significance in this matter.

5. The Hindu and Parsee women of the Bombay area follow rules, involving a menstrual isolation of 3 + 1 days only. The very scanty data available (25 cases only) show a much lower incidence of cancer of the cervix on Parsees. This might signify that so short a post-menstrual period does not affect the matter one way or the other, and this difference in liability to cancer must then be due to other factors. The similar social incidence in both married and single women in England and Wales shows that such factors exist.

6. Cancer of the uterus appears to be prevalent in some peoples (Hindus, Chinese) among whom phimosis and cancer of the penis are also common, while the Moslem women of India show a lower incidence. But any attribution of this difference to the practice of circumcision by Moslems is very doubtful in view of the similar low incidence on peoples (Parsees, Indian Christians, possibly some Dutch) in whom this factor is absent. But the numerical data on this matter are still quite inadequate.

7. The only numerical data on the social incidence of cancer of the uterus appear to be those from Bavaria 40 years ago, and from England and Wales, after the last census, in 1930–32. The liability to cancer of the uterus increases with descent in the social scale, and, in England and Wales, this is true of both married and single women.

8. The unavoidable mixture, in large-scale statistics, of cancers of the cervix and of the corpus uteri, is unfortunate, as these two forms probably differ in etiology. The difference of 8–10 years in the maximum age-incidence of the two sometimes enables suggestions to be made of the relative proportions of the two in numerical data.

9. The unique data of the Registrar-General upon the social incidence of cancer of the uterus in married and single women suggests a factor less closely connected with sexual functions than are those discussed above in connection with various rituals.

10. The data collected in this paper suggest the existence of two factors which may increase the incidence of cancer of the uterus, namely :

(1) A factor which is opposed by the Jewish practice of abstention from intercourse during most of the first half of the ovulatory cycle ; and

(2) A factor which is intensified in both married and single women by descent in the economic scale.

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A STATISTICAL REPORT ON 2529 CASES OF CANCER OF THE BREAST.

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Published for the Clinical Cancer Research Committee of the British Empire Cancer Campaign.

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IN 1938–39 the Clinical Cancer Research Committee of the British Empire Cancer Campaign carried out a clinical survey of all cases of cancer seen in the hospitals, both Voluntary and L.C.C. in the Administrative County of London. This was done by means of questionnaires, one of which was filled in by the Registrars in the various hospitals for each patient and returned to the Clinical Cancer Research Committee for record. The work was interrupted by the outbreak of war after it had been in progress for 17 months. By this time 15,200 cases of cancer had been registered, of which 2529 (16·6 per cent) were cancer of the breast. These patients have now been followed up for five years or more and the records analysed. The full report will be published in due course by the British Empire Cancer Campaign, but the main findings are here presented.

There were 2152 primary and 377 recurrent cases. 23 of the 2152 primary cases were males (1·07 per cent) and 2129 were females (98·93 per cent)—the same ratio as found in other large series.

PRIMARY CASES—FEMALES (2129).

The percentage of single, married and widowed amongst the 2129 females is shown below, together with the corresponding figures from Lane-Claypon's (1926) report on cancer of the breast, in which figures for 508 cancer patients were compared with 509 controls.

Civil State.

	British Empire Cancer Campaign, 2129 cancer cases.		Lane-Claypon.			
			508 cancer cases.		509 control cases.	
	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Single	471	22·1	116	22·8	87	17·1
Married	1236	58·0	292	57·5	321	63·1
Widowed	422	19·8	100	19·7	101	19·8

The difference between the percentages of single women in the cancer series and in the control series, $5{\cdot}7 \pm 2{\cdot}5$, is statistically significant. It will be seen that the percentages in the B.E.C.C. series are almost the same as those in Lane-Claypon's cancer series. The Registrar-General (1938) concludes from an analysis of 13,298 deaths from cancer of the breast that "at each age above 35 single women suffered considerably higher mortality than others from this cause."

Age Distribution.

Age distribution.	Single.	Married and widowed.	Total.
15–24	3	1	4
25–34	19	40	59
35–39	23	99	122
40–44	55	147	202
45–49	61	217	278
50–54	80	214	294
55–59	53	219	272
60–64	57	223	280
65–69	48	217	265
70–74	44	141	185
75–79	17	100	117
80–84	7	30	37
85 and over	4	10	14
Mean age	$55{\cdot}6 \pm 0{\cdot}6$	$57{\cdot}4 \pm 0{\cdot}3$	$57{\cdot}0 \pm 0{\cdot}4$
Standard deviation	$12{\cdot}8 \pm 0{\cdot}4$	$12{\cdot}5 \pm 0{\cdot}2$	$12{\cdot}6 \pm 0{\cdot}2$

Difference between mean ages of single and married $57{\cdot}4–55{\cdot}6 = 1{\cdot}8 \pm 0{\cdot}66$.

The mean age is 5–6 years higher than those given by Lane-Claypon (1926) and Wainwright (1931), due to the fact that the two latter series include only cases coming to hospital for operation, whereas the B.E.C.C. series includes all cases seen, whether operable or not. The youngest patient in the series was aged 15, and survived 5 years after enucleation of a tumour of 3 years' standing, which proved on histological examination to be an early adenocarcinoma. The oldest patient was aged 90, and died of recurrence 5 months after amputation of the breast for an ulcerating growth, proved histologically to be an adenocarcinoma.

Heredity.

A history of the patient's father having died of cancer of any region was given by 4·5 per cent of all patients, and of death of the mother from cancer of any region by 8·3 per cent, from cancer of the breast by 3·0 per cent. 382 patients gave histories of 452 parents or siblings having suffered from cancer, so that in 70 instances (3·3 per cent) more than one relative was affected. There were 17 instances of both parents having died of cancer amongst 1589 patients of known family history, or 1 in 93; the expectation of this occurring by chance is 1 in 151; Stocks and Karn (1933) found the expected frequency of this event for cancer of all regions to be 1 in 180, the numbers involved being 364 fathers and 323 mothers. As no information about family history was available for 25·4 per cent of the patients, it is impossible to draw definite conclusions from the figures.

Relation to the Menopause.

30·4 per cent of the single women and 25·5 per cent of the married had not reached the menopause, and in 2·6 per cent and 3·4 per cent respectively it was in progress when the disease first appeared. In 18·7 per cent and 18·3 per cent the question was not answered. Lane-Claypon (1926) has shown that the mean age of cessation of the catamenia in a series of 328 patients with cancer of the breast was not significantly different from that of a control series of 332 women.

Children and Miscarriage.

The percentage of childless marriages amongst 1658 married and widowed women was 16·1; Lane-Claypon found that 18·4 per cent of 261 women with cancer of the breast and 12·5 per cent of 280 control patients were childless. She concluded from a statistical study of the data by Major Greenwood that "the fertility of the cancer patients is definitely less than that of the control patients." As no information on this point was available for 13·5 per cent of patients in our series, it is impossible to say whether or not the figures support Lane-Claypon's conclusions. For the same reason we can give no reliable figures

	Number of cases.	Per cent.
Lump in breast	1649	77·4
Pain in breast	213	10·0
"Eczema" of skin of breast	57	2·7
Retraction of nipple	41	2·0
Referred pain	34	1·6
Symptoms due to metastases	34	1·6
Bloody discharge from nipple	26	1·2
Discharge from nipple	20	1·0
Lump in axilla	18	0·8
Loss of weight and general weakness	2	0·1
No symptoms. Tumour discovered during routine examination	34	1·6
Not stated	1	0·05

of the proportion of women with cancer of the breast who had or had not nursed their children. Lane-Claypon found that 14·6 per cent of the children of women who afterwards developed cancer of the breast were not nursed and 7·4 per cent of the children of those in the control series—a statistically significant difference.

First Symptom.

A lump in the breast was the first symptom complained of in 77·4 per cent of the patients, pain in the breast in 10·0 per cent, and other symptoms as shown in the table (see p. 214).

The 34 patients in whom the first symptoms were those due to metastases were found to have the following :

	Number of cases.
Symptoms due to enlarged axillary nodes	2
,, ,, ,, supraclavicular nodes	3
,, ,, ,, lymph nodes elsewhere	1
,, ,, metastases in vertebral column	13
,, ,, ,, liver	5
,, ,, ,, lung	3
,, ,, ,, brain	3
,, ,, ,, skull	2
,, ,, ,, femur	2

Bloody discharge from the nipple was the first symptom in 26 patients. The histological reports on these tumours were : spheroidal cell carcinoma 8, duct carcinoma 7, adenocarcinoma 4, carcinoma, type not specified 1, squamous cell carcinoma 1, not examined histologically 5.

Interval from First Symptom to First Consulting a Doctor.

	Number of cases.	Per cent.
1 month and under	508	
1–2 months	266	44·1
2–3 ,,	164	
3–4 ,,	117	
4–6 ,,	208	15·3
6–9 ,,	136	
9–12 ,,	190	
12–18 ,,	83	
18–24 ,,	119	33·3
24–48 ,,	85	
Over 48 months	97	
Not stated	156	7·3

These figures show that there was serious delay on the part of 48·6 per cent of the patients in seeking medical advice, and in 8·6 per cent the interval was 2–4 years or more.

Advice and Treatment before Admission to Hospital.

	Number of cases.
No doctor consulted prior to coming to hospital	293
Referred to hospital without delay	1472
Advised to go to hospital, but delayed going there	99
Treated symptomatically for periods up to 3 months before reference	33
Treated symptomatically for periods of more than 3 months before reference	88
Reassured and told condition not serious	66
Not stated	78
Refused the treatment advised	22

Of the 1836 patients who consulted a doctor, 85·6 per cent were referred to hospital at once, though 5·4 per cent of these delayed in following this advice or perhaps had to wait some weeks for a vacant bed; 1·8 per cent were kept under observation and symptomatic treatment for periods up to 3 months, and 4·8 per cent for longer periods, but many of the patients in the latter group were in an advanced stage of the disease, or were unsuitable for operative treatment by reason of poor general condition. 3·6 per cent of the patients were reassured and told that there was nothing serious the matter. Only 1·0 per cent refused the treatment advised at hospital.

Findings on Examination in Hospital.

Location of tumour.	Number of cases.	Per cent.
Left breast	1114	52·3 ± 1·09
Right breast	1005	47·2
Both breasts	6	0·3
Not stated	4	0·2

Busk and Clemmesen (1947), investigating the site of 4139 Danish cases, found that the proportions were 111 on the left to 100 on the right—almost exactly the same figures as in our series—and that the difference was statistically significant. Ewing (1940) states that the disease appears to originate simultaneously in both breasts in 1·5 per cent, Harrington (1938) found simultaneous carcinoma in 1·0 per cent and bilateral carcinoma occurring at different times in 4·5 per cent of 4628 cases. In the present series 60, or 2·8 per cent, were found to have growths in both breasts, but 54 of these were considered to be metastases from a pre-existing primary in the other breast, and 6 to be examples of simultaneous carcinoma:

Site in breast.	Number of cases.	Per cent.
Upper and outer quadrant	916	43·0
,, inner ,,	283	13·3
Central, behind nipple	235	11·0
Lower and outer quadrant	204	9·6
,, inner ,,	94	4·4
Diffuse, too advanced to identify the primary site	324	15·2
Not stated	74	3·5

There was 1 patient who had 2 separate growths in the upper inner and lower outer quadrants respectively, making a total of 2130 tumours.

Size of tumour.	Number of cases.	Per cent.
Small—up to 4 sq. cm.	216	10·1
Medium—4–20 sq. cm.	717	33·7
Large—20–100 sq. cm.	752	35·4
Massive—over 100 sq. cm.	193	9·1
Not stated	251	11·8

Ulceration of skin.	Number of cases.	Per cent.
Present	439	20·6
Not present	1614	75·8
Not stated	76	3·6

Skin Metastases. Clinical Involvement of Lymph Nodes.

	Number of cases.	Per cent.
Skin nodules present	245	11·5
,, ,, not present	1789	84·1
Not stated	95	4·5
Axillary lymph nodes involved :		
Unilateral	1195	56·1
Bilateral	76	3·6
No lymph nodes involved	812	38·2
Not stated	46	2·2
Supraclavicular nodes also involved :		
Unilateral	189	9·0
Bilateral	31	1·5

These figures include all cases in which the lymph nodes were palpable; in many of them other complications such as fixation to skin or distant metastases were also present.

Remote Metastases.

	Number of cases.		Per cent.
None found on clinical examination	1075		
,, ,, radiological examination	749	1824	85·7
Metastases present	219		10·3
Not stated	86		4·0

Sites of Metastases in 219 Patients (Multiple in Many Cases).

Mediastinal lymph nodes	27
Lymph nodes, other than regional nodes	7
Skin and subcutaneous tissues	6
Lungs and pleurae	86
Liver and abdominal organs	49
Brain	8
Skull, spine, ribs and sternum	69
Bones of pelvis	24
Bones of extremities	30

This table does not include metastases in the supraclavicular lymph nodes, the figures for which are shown above.

Condition of the Opposite Breast.

	Number of cases.	Per cent.
A cancerous growth was present	60	2·8
Opposite breast had been amputated previously for cancer	23	1·1
Nodular mastitis or cysts present	74	3·5
Opposite breast has been treated surgically or by radiotherapy for mastitis	10	0·5
Normal	1866	87·6
Not stated	96	4·5

Of the 60 cases with growths in both breasts, 6 were considered to be true simultaneous growths, and 54 to be metastases from a primary in the other breast. The 23 cases in which the opposite breast had been amputated for cancer previously may have been either recurrences or second primaries.

General Condition.

	Number of cases.	Per cent.
Obese; general condition not noted	13	0·6
Good condition; no weight loss	1203	56·5
Fair condition; moderate weight loss (up to 2 st.)	468	21·9
Poor condition; considerable weight loss (over 2 st.)	189	8·9
Emaciated	33	1·6
Moribund	27	1·3
Not stated	196	9·2

Other Co-existing Diseases.

Was suffering from diabetes	23	1·1
,, ,, ,, cardiovascular disease	71	3·4
,, ,, ,, pulmonary tuberculosis	5	0·2
,, ,, ,, mental affection (usually senile dementia)	26	1·2

Clinical Stages.

The cases were grouped into 4 clinical stages according to the presence or absence of clinical involvement of lymph nodes, invasion of adjacent tissues and distant metastases, as follows :

Stage I : Growth confined to breast ; no involvement of axillary lymph nodes nor infiltration of skin or muscles.

Stage II : Growth confined to breast ; axillary lymph nodes involved, but no infiltration of skin or muscles.

Stage III*a* : Growth infiltrating skin or muscle, or both, but no involvement of axillary lymph nodes.

Stage III*b* : As in III*a*, but axillary lymph nodes involved.

Stage IV : Remote metastases present. Cases with involvement of the supraclavicular lymph nodes were placed in this stage.

Not staged for lack of data.

Arranged in accordance with this system the numbers in each stage were :

	Number of cases.	Per cent.
Stage I	517	24·3
,, II	400	18·8
,, III*a*	233	10·9
,, III*b*	533	25·0
,, IV	352	16·6
Not staged	94	4·4

In the case of patients who underwent operation, or died soon after admission, the operation and pathological findings where available were used for correcting the staging. Patients not operated on, or who did not die in hospital, were placed in the appropriate stage according to the clinical evidence of extension of the growth and the presence of metastases. The table below indicates when the staging is based on clinical evidence alone and when on histological findings.

Stage I.—Confined to breast ; lymph nodes not involved or doubtful.

	Number.
No lymph nodes involved clinically	95
,, ,, ,, histologically	111
Involvement of lymph nodes doubtful clinically	6
With slight adhesion to skin ; no lymph nodes involved clinically	118
,, ,, ,, ,, ,, ,, histologically	148
,, ,, ,, involvement of lymph nodes doubtful clinically	7
Total number of cases	485

Stage II.—Confined to breast; axillary lymph nodes involved.

	Number.
Axillary lymph nodes involved clinically	44
,, ,, ,, histologically	120
Slight adhesion to skin, with axillary nodes involved clinically	89
,, ,, ,, ,, ,, ,, histologically	214
Total number of cases	467

Stage IIIa.

Fixed to skin or ulcerated; *no* nodes involved clinically	81
,, ,, ,, ,, ,, histologically	33
Adherent to pectoral fascia; *no* nodes involved clinically	5
,, ,, ,, ,, ,, histologically	8
Fixed to skin and pectoral fascia; *no* nodes involved clinically	46
,, ,, ,, ,, ,, ,, histologically	51
Total number of cases	224

Stage IIIb.

Fixed to skin *with* axillary nodes involved clinically	183
,, ,, ,, ,, ,, histologically	125
Adherent to pectoral fascia *with* nodes involved clinically	5
,, ,, ,, ,, histologically	8
Fixed to skin and pectoral fascia; *no* nodes involved clinically	109
,, ,, ,, ,, ,, histologically	108
Total number of cases	538

Stage IV.—Remote metastases present.

Supraclavicular lymph nodes alone involved	144
Remote metastases present	218
Total number of cases	362

Not staged for lack of data 53

Comparison of Clinical and Final Staging.

	Clinical. Number of cases.	Final. Number of cases.	Clinical. Per cent.		Final. Per cent.	
Stage I	517	485	24·3		22·8	
,, II	400	467	18·8		21·9	
,, III*a*	233	224	10·9	35·9	10·5	35·7
,, III*b*	533	538	25·0		25·2	
,, IV	352	362	16·6		17·1	
Not staged	94	53	4·4		2·5	

The 5-year survival rates of patients treated by radical mastectomy who were classified in stages I and II on clinical evidence and on histological evidence respectively were compared, with the following results :

		Number.	Survived.	Per cent.	Difference.
Stage I .	*No lymph nodes involved :*				
	On clinical evidence . .	70 .	37 .	52·9	10·1 ± 6·7
	On histological evidence .	235 .	148 .	63·0	
„ II .	*Lymph nodes involved :*				
	On clinical evidence . .	50 .	21 .	42·0	3·6 ± 7·5
	On histological evidence .	302 .	116 .	38·4	

These differences are not statistically significant.

Methods of Treatment and 5-year Results (see Table, pp. 222, 223).

All deaths from any cause within one month of an operation, whether radical or palliative, were counted as "operation fatalities." In the case of radiotherapeutic treatment, those cases in which death appeared to have been accelerated by the effects of radiotherapy have been classified as "died from effects of treatment," regardless of the time which had elapsed since treatment was completed.

There were 4 "operation fatalities" following the insertion of radium needles ; 1 of these was due to shock, 1 to post-anaesthetic pneumonia, 1 to pulmonary embolism and 1 to suppurative pericarditis. Six patients were classified as "died from effects of treatment" ; 1 from pulmonary embolism 2 weeks after the insertion of radium needles ; 1, who was aged 70, appears to have died from radiation sickness shortly after the termination of a course of X-ray treatment, and 4 from radio-necrosis. There was also a patient who died from late radio-necrosis of the rib 7½ years after the operation, making 11 cases in all.

Five-year Follow-up Results for All Cases.

	First year.	Second year.	Third year.	Fourth year.	Fifth year.	Totals.		
Operation fatalities . .	34 .	— .	— .	— .	— .	34		
Died under treatment . .	5 .	1 .	— .	— .	— .	6	1382	
„ with cancer . .	551 .	326 .	178 .	146 .	79 .	1280		
„ without cancer . .	10 .	11 .	13 .	9 .	19 .	62		
Alive and well . . .	.. .	.. .	.. .	.. .	447			2129
„ with cancer . .	.. .	.. .	.. .	.. .	109	..	664	
„ state unknown . .	.. .	.. .	.. .	.. .	108			
Untraced . . .	.. .	.. .	.. .	.. .	83 .	..	83	
Total died yearly . .	600 .	338 .	191 .	155 .	98 .	1382		
Percentage of all cases .	28·2 .	15·9 .	9·0 .	7·3 .	4·6			

The five-year survival rate for all cases was 31·2 per cent.

Of the 664 survivors, 47 are known to have died of cancer, and 13 without cancer in the sixth and subsequent years.

Methods of Treatment and 5-Year Results.

Radical mastectomy alone or with Radiotherapy.	Number.	Operation fatalities.	Died from effects of treatment.	Survived 5 years.	Died with cancer.	Died without cancer.	Not traced.	Operation fatalities per cent.
Radical mastectomy	703	22	—	312	299	22	48	3·1
Radical mastectomy followed by H.V. X-rays	334	—	—	134	182	6	12	—
Radical mastectomy preceded by H.V. X-rays	27	3	—	6	17	1	—	11·1
Radical mastectomy preceded and followed by H.V. X-rays	12	—	—	2	10	—	—	—
Radical mastectomy with implantation of radium	15	2	—	7	6	—	—	13·3
Radical mastectomy with implantation of radium followed by X-rays or surface radium	5	—	—	3	2	—	—	—
	1096	27		464	516	29	60	2·5
Local mastectomy alone or with Radiotherapy.								
Local mastectomy*	133	2	—	52	58	16	5	1·5
Local mastectomy followed by H.V. X-rays or surface radium	85	—	—	37	43	3	2	—
Local mastectomy with implantation of radium in lymph nodes	15	1	—	3	11	—	—	6·6
	233	3		92	112	19	7	1·3
Radium alone or with local surgery.								
Interstitial radium alone	74	4	1	9	56	2	2	6·8
Surface radium alone	11	—	—	1	9	—	1	—
Teleradium alone	11	—	—	2	8	1	—	—
Teleradium with H.V. X-rays	8	—	—	—	7	1	—	—
Interstitial radium followed by surface radium	6	—	—	—	4	—	2	—
Interstitial radium followed by X-rays to lymph nodes	10	—	—	2	8	—	—	—

* Axillary lymph nodes excised also in 83 patients.

	Number.	Operation fatalities.	Died from effects of treatment.	Survived 5 years.	Died with cancer.	Died without cancer.	Not traced.	Operation fatalities per cent.
Teleradium followed by interstitial radium	1	—	—	—	1	—	—	—
Excision of tumour followed by implantation of radium	45	—	—	26	14	1	4	—
Interstitial radium followed by local mastectomy	7	—	—	3	4	—	—	—
Teleradium followed by local or radical mastectomy 1 year or more later	2	—	—	1	1	—	—	—
	175	4	1	44	112	5	9	
X-rays alone or with radium implantation and/or local surgery.								
H.V. X-rays alone	270	—	3	19	241	5	2	1·1
H.V. X-rays followed by interstitial radium	59	—	2	15	41	1	—	3·4
H.V. X-rays followed by local mastectomy	32	—	—	9	22	1	—	—
Local mastectomy preceded and followed by H.V. X-rays	2	—	—	1	1	—	—	—
H.V. X-rays followed by interstitial radium and local excision	6	—	—	1	4	1	—	—
Local excision of tumour followed by H.V. X-rays	21	—	—	9	10	1	1	—
Local excision of tumour preceded and followed by H.V. X-rays	3	—	—	2	1	—	—	—
	393		5	56	320	9	3	1·3
Not treated by surgery or radiotherapy	232	—	—	8	220	—	4	—
Grand totals	2129	34	6	664	1280	62	83	
Per cent	100·0	1·6	0·3	31·2	60·1	2·9	3·9	

Analysis of Five-year Results by Methods of Treatment and Stages.

Surgical or Combined Methods.		Stage I.	Stage II.	Stage IIIa.	Stage IIIb.	Stage IV.	Not staged.	Total.
Radical mastectomy	*No.*	*236*	*216*	*72*	*163*	*15*	*1*	*703*
Known survivors		144	88	39	41	—	—	312
Per cent survived		61·0	40·7	54·2	25·2	0·0	0·0	44·4
Per cent of traced cases		68·2	43·6	59·1	25·6	0·0	0·0	47·6
Radical mastectomy combined with radiotherapy	*No.*	*69*	*136*	*26*	*105*	*19*	*38*	*393*
Known survivors		41	49	11	31	4	16	152
Per cent survived		59·4	36·0	42·3	29·5	21·2	42·1	38·7
Per cent of traced cases		64·1	37·1	44·0	30·1	21·2	42·1	39·9
Local mastectomy	*No.*	*61*	*19*	*22*	*21*	*9*	*1*	*133*
Known survivors		34	4	9	4	—	1	52
Per cent survived		55·7	21·2	40·9	19·0	0·0	100·0	39·1
Per cent of traced cases		59·6	21·2	40·9	19·0	0·0	100·0	40·6
Local mastectomy combined with radiotherapy	*No.*	*26*	*23*	*12*	*24*	*8*	*7*	*100*
Known survivors		16	8	6	4	—	6	40
Per cent survived		61·5	34·8	50·0	16·6	0·0	85·7	40·0
Per cent of traced cases		64·0	34·8	50·0	17·4	0·0	85·7	40·8
Radium alone or with local surgery.								
Interstitial radium alone	*No.*	*12*	*5*	*11*	*29*	*17*	—	*74*
Known survivors		3	—	3	3	—	—	9
Per cent survived		25·0	0·0	27·3	10·3	0·0	—	12·2
Per cent of traced cases		30·0	0·0	27·3	10·3	0·0	—	12·5
Interstitial radium with surface radium, teleradium or X-rays	*No.*	*3*	*5*	*2*	*5*	*2*	—	*17*
Known survivors		—	2	—	—	—	—	2
Per cent survived		0·0	40·0	0·0	0·0	0·0	—	11·8
Surface radium or teleradium with/without X-rays	*No.*	—	*2*	*9*	*8*	*11*	—	*30*
Known survivors		—	—	2	—	1	—	3
Per cent survived		—	0·0	22·2	0·0	9·9	—	10·0
Excision of tumour followed by implantation of radium	*No.*	*25*	*10*	*4*	*3*	*2*	*1*	*45*
Known survivors		14	6	2	3	—	1	26
Per cent survived		56·0	60·0	50·0	100·0	0·0	100·0	57·8
Per cent of traced cases		63·6	66·6	50·0	100·0	0·0	100·0	63·4
Interstitial radium followed by local mastectomy	*No.*	*1*	*1*	*2*	*3*	—	—	*7*
Known survivors		—	—	2	1	—	—	3
Per cent survived		0·0	0·0	100·0	33·3	—	—	42·8
Teleradium followed by local or radical mastectomy	*No.*	—	*1*	—	*1*	—	—	*2*
Known survivors		—	—	—	1	—	—	1
Per cent survived		—	0·0	—	100·0	—	—	50·0

X-rays alone or with radium and/or local surgery.	Stage I.	Stage II.	Stage IIIa.	Stage IIIb.	Stage IV.	Not staged.	Total.
H.V. X-rays alone . . *No.*	*18*	*22*	*25*	*89*	*114*	*2*	*270*
Known survivors . . .	5	3	3	5	2	1	19
Per cent survived . . .	27·7	13·6	12·0	5·6	1·8	50·0	7·0
Per cent of traced cases . .	27·7	13·6	12·0	5·7	1·8	50·0	7·1
H.V. X-rays followed by interstitial radium . . *No.*	*7*	*5*	*11*	*26*	*10*	—	*59*
Known survivors . . .	3	1	4	7	—	—	15
Per cent survived . . .	42·8	20·0	36·4	26·9	0·0	—	25·4
H.V. X-rays followed by interstitial radium and local excision . . . *No.*	*1*	—	*2*	*2*	*1*	—	*6*
Known survivors . . .	1	—	—	—	—	—	1
Per cent survived . . .	100·0	—	0·0	0·0	—	—	16·6
H.V. X-rays followed by local mastectomy . . *No.*	*4*	*5*	*7*	*15*	*3*	—	*34*
Known survivors . . .	3	1	2	3	1	—	10
Per cent survived . . .	75·0	20·0	28·6	20·0	33·3	—	29·4
Local excision of tumour followed by H.V. X-rays . . *No.*	*16*	*4*	—	*2*	*1*	*1*	*24*
Known survivors . . .	9	2	—	—	—	—	11
Per cent survived . . .	56·2	50·0	—	0·0	0·0	0·0	45·8
Per cent of traced cases . .	60·0	50·0	—	0·0	0·0	0·0	47·8
Not treated by surgery or radiotherapy. *No.*	*6*	*13*	*19*	*42*	*150*	*2*	*232*
Known survivors . . .	1	—	5	2	—	—	8
Per cent survived . . .	16·6	0·0	26·3	4·9	0·0	0·0	3·4

Of the 8 surviving patients in this group, 2 died of cancer in the 6th and 8th years respectively, 4 are still alive with ulcerating growths; the diagnosis of malignancy has been changed in 1, and no details of the present condition of the other are known.

	Stage I.	Stage II.	Stage IIIa.	Stage IIIb.	Stage IV.	Not staged.	Total.
Total results . . . . *No.*	*485*	*467*	*224*	*538*	*362*	*53*	*2129*
Known survivors . . .	274	170	85	102	8	25	664
Per cent survived . . .	56·5	36·4	37·9	19·0	2·2	47·2	31·2

Truscott (1947) gives the following percentage survival rates after five years for all types of treatment of 836 cases, the cases being staged on the same principles as in the above series.

Stage.	Number.	Percentage alive.
I	250	46·0
II	484	17·3
III*a*	24	8·3
III*b*	78	7·7

According to Table X in Truscott's paper there were 59·6 per cent known 5-year survivors of 114 followed-up stage I cases treated by surgery alone, and 25·8 per cent of 163 followed-up stage II cases. The corresponding figures in the B.E.C.C. series are: 68·2 per cent of 210 followed-up stage I cases, and 43·6 per cent of 202 followed-up stage II cases.

Analysis of 5-year Results by Ages in Relation to Methods of Treatment.

Age group.	Radical mastectomy alone.			Radical mastectomy with radiotherapy.			Local mastectomy alone.		
	Total number.	Known survivors.	Per cent.	Total number.	Known survivors.	Per cent.	Total number.	Known survivors.	Per cent.
15–	1	—	0·0	1	1	100·0	2	2	100·0
25–	17	6	35·3	25	5	20·0	2	1	50·0
35–	132	64	48·5	90	33	36·6	8	5	62·5
45–	221	106	48·0	145	63	43·4	22	9	40·9
55–	210	88	41·9	79	30	37·9	27	10	37·0
65–	107	43	40·2	50	19	38·0	46	16	34·8
75–	14	5	35·7	3	1	33·3	26	9	34·1
Totals	702	312	44·4	393	152	38·7	133	52	39·1
	$\chi^2 = 4·29$ $P < 0·70 > 0·50$			$\chi^2 = 5·81$ $P < 0·50 > 0·30$			$\chi^2 = 5·44$ $P < 0·50 > 0·30$		

Similar calculations for the series of cases treated by local mastectomy combined with radiotherapy, by radium with or without local surgery, and by X-rays with or without local surgery showed, as the above figures do, that the variations in survival rates of the different age-groups are not statistically significant.

Radical Mastectomy: 5-year Results by Duration of Symptoms.

The duration of symptoms at the time of operation is tabulated for 368 patients who had radical mastectomy, and for 177 who had radical mastectomy with radiotherapy. The estimates of the duration of symptoms are based on the patients' statements.

Duration of Symptoms.	Radical mastectomy.		Radical mastectomy with radiotherapy.		Totals.
	Number.	Known survivors.	Number.	Known survivors.	
1 month and under	43	20	16	4	
1–3 months	101	52	46	20	
3–6 ,,	81	40	44	20	
Total under 6 months	225	112 (50%)	106	44 (41%)	331–156 = 47·1%
6–12 months	56	19	27	11	
Over 12 months	77	29	41	20	
Total over 6 months	133	48 (36·1%).	68	31 (45·6%).	201–79 = 39·6%
Duration not known	11	4	3	—	
Differences in survival rate	—	13·9 ± 5·3.	—	4·1 ± 7·7.	7·5 ± 4·4

These figures show that in the early cases radical mastectomy alone gives the best survival rate, but that in cases of longer standing the survival rate was improved by the use of radiotherapy in addition. The difference in percentage is statistically significant for radical mastectomy, but not for combined treatment.

Analysis of 5-year Results by Site of the Growth in the Breast.

	Radical mastectomy.								
	Stage I. 229.			Stage II. 207.			All stages. 668.		
	Number.	Known survivors.	Per cent.	Number.	Known survivors.	Per cent.	Number.	Known survivors.	Per cent.
Upper and outer	112	73	65·2	123	56	45·5	354	173	48·9
„ „ inner	56	34	60·7	24	6	25·0	106	48	45·3
Lower and outer	35	20	57·1	28	12	42·8	90	38	42·2
„ „ inner	8	4	50·0	6	2	33·3	31	15	48·3
Central	18	11	61·1	26	11	42·3	87	37	42·5
Totals	229	142	62·2	207	87	42·0	668	311	46·6
	$\chi^2 = 1·51$ $P < 0·90 > 0·80$			$\chi^2 = 3·80$ $P < 0·50 > 0·30$			$\chi^2 = 2·34$ $P < 0·70 > 0·50$		

It will be seen from the values of χ^2 and P that none of these variations from the mean is statistically significant.

Radical mastectomy: effect on prognosis of slight attachment to the skin.

In the final staging on p. 219 it will be seen that cases in stages I and II with slight attachment to the skin are separated from those in which there was no attachment. This enabled the survival rate after radical mastectomy alone and combined with radiotherapy to be worked out separately, with the following result:

		Number.	Survived.	Per cent.	Difference.
Stage I	Not adherent	128	73	57·0	$6·3 \pm 5·7$
	Adherent	177	112	63·3	
Stage II	Not adherent	122	46	37·7	$1·9 \pm 5·4$
	Adherent	230	91	39·6	

These differences are not statistically significant.

Radical mastectomy: effect on prognosis of coincident pregnancy.

There were 10 patients who were pregnant at the time of discovery of the growth in the breast, which was found to be in a relatively advanced stage in all cases. Only 2 of them were treated by radical mastectomy, 1 of whom survived 5 years and 1 died of cancer in the 5th year.

Radical mastectomy: effect on prognosis of exploratory incision.

Data on this point were recorded for 1050 cases of radical mastectomy, with or without radiotherapy in addition:

	Number.	Known Survivors.	Per cent.
Exploratory incision followed by mastectomy immediately	95	58	61·1
Exploratory incision followed by mastectomy after an interval	65	38	58·5
Mastectomy without exploratory incision	536	210	39·3
Not stated	354	138	39·0

The differences in survival rate between those in whom an exploratory incision was made (160), and those in whom this was not done (536), are statistically significant and are probably due to the fact that the former were early cases in which the diagnosis was in doubt. The differences between the two groups in which an exploratory incision was made are not statistically significant.

Ulceration of skin : effect on prognosis (all treatments combined).

The survival rate of cases with fixation to the skin and with actual ulceration were worked out separately. The figures are :

No lymph nodes involved :	Number.	Survived.	Per cent.	Difference.
Growth fixed to skin	41	22	53·7	$24 \cdot 9 \pm 9 \cdot 4$
Skin ulcerated	73	21	28·8	
Lymph nodes involved :				
Growth fixed to skin	104	24	23·1	$7 \cdot 4 \pm 4 \cdot 8$
Skin ulcerated	204	32	15·7	

Ulceration has a serious effect on prognosis, but the difference in the survival rate is only statistically significant in those cases where no lymph nodes are involved.

Estimation of Survival after Treatment.

Dr. Stocks, to whom this question was referred, advised that unless the follow-up of cases makes it possible to assign accurately every death either to cancer on the one hand or to intercurrent causes on the other, the only sound method of dealing with the duration of survival is an actuarial one, which means calculating from a life-table the total months which would be lived in the period of observation by a group of people in the general population having the same sex-age distribution as the group of patients dealt with. This gives the mean number of months expected to be lived during the five years by each group. The mean number of months actually lived is then calculated and expressed as a percentage of the normal expected for that group, making allowances for cases followed up for less than five years.

English Life Table No. 10 (1930–32) was used for ascertaining the expectation of life.

Radical mastectomy alone.	Stage I.	Stage II.	Stage IIIa.	Stage IIIb.
Number of cases of known duration	*210*	*198*	*68*	*158*
Mean number of months lived in 5 years from onset — Maximum possible	60·00	60·00	59·12	59·77
Mean number of months lived in 5 years from onset — Expected	57·20	57·16	56·02	57·04
Mean number of months lived in 5 years from onset — Actual	52·01	43·28	47·59	39·02
Per cent of Expected	91·03	75·72	84·95	68·41
Radical mastectomy with post-operative radiation.				
Number of cases of known duration	*55*	*116*	*21*	*78*
Mean number of months lived in 5 years from onset — Maximum possible	60·00	60·00	59·43	60·00
Mean number of months lived in 5 years from onset — Expected	57·95	57·89	56·78	57·36
Mean number of months lived in 5 years from onset — Actual	51·16	40·53	47·81	41·73
Per cent of Expected	88·28	70·01	84·20	72·75

	Stage I.	Stage II.	Stage IIIa.	Stage IIIb.
Radical mastectomy with pre-operative radiation.				
Number of cases of known duration	*3*	*7*	*3*	*18*
Mean number of months lived in 5 years from onset — Maximum possible	60·00	60·00	60·00	60·00
Mean number of months lived in 5 years from onset — Expected	57·63	58·57	57·63	57·63
Mean number of months lived in 5 years from onset — Actual	46·66	32·00	53·66	37·77
Per cent of Expected	80·93	54·64	93·11	65·54
Radical mastectomy with implantation of radium.				
Number of cases of known duration	*3*	*6*	*2*	*16*
Mean number of months lived in 5 years from onset — Maximum possible	60·00	60·00	60·00	60·00
Mean number of months lived in 5 years from onset — Expected	59·16	58·02	48·04	56·43
Mean number of months lived in 5 years from onset — Actual	60·00	38·00	35·50	45·83
Per cent of Expected	101·42	65·49	73·90	81·22
Local mastectomy alone.				
Number of cases of known duration	*56*	*18*	*21*	*20*
Mean number of months lived in 5 years from onset — Maximum possible	60·00	60·00	60·00	60·00
Mean number of months lived in 5 years from onset — Expected	53·08	52·88	53·27	53·07
Mean number of months lived in 5 years from onset — Actual	51·82	40·28	46·38	37·15
Per cent of Expected	97·63	76·17	87·07	70·00
Local mastectomy with post-operative radiation.				
Number of cases of known duration	*23*	*22*	*12*	*23*
Mean number of months lived in 5 years from onset — Maximum possible	60·00	60·00	60·00	60·00
Mean number of months lived in 5 years from onset — Expected	54·64	56·49	54·31	55·09
Mean number of months lived in 5 years from onset — Actual	55·22	42·09	49·33	39·39
Per cent of Expected	101·06	74·51	90·83	71·50
Interstitial radium alone or with surface radium.				
Number of cases of known duration	*13*	*10*	*12*	*32*
Mean number of months lived in 5 years from onset — Maximum possible	59·08	60·00	57·00	60·00
Mean number of months lived in 5 years from onset — Expected	52·56	55·53	50·59	55·42
Mean number of months lived in 5 years from onset — Actual	41·38	32·80	37·83	29·16
Per cent of Expected	78·73	59·07	74·78	52·62
Interstitial radium with local surgery.				
Number of cases of known duration	*23*	*10*	*6*	*6*
Mean number of months lived in 5 years from onset — Maximum possible	60·00	60·00	60·00	60·00
Mean number of months lived in 5 years from onset — Expected	57·12	58·54	58·16	55·96
Mean number of months lived in 5 years from onset — Actual	49·91	47·20	55·17	47·50
Per cent of Expected	87·38	80·63	94·86	84·88
X-rays alone or with interstitial radium.				
Number of cases of known duration	*25*	*25*	*34*	*112*
Mean number of months lived in 5 years from onset — Maximum possible	60·00	60·00	60·00	60·00
Mean number of months lived in 5 years from onset — Expected	52·08	52·18	53·55	54·87
Mean number of months lived in 5 years from onset — Actual	41·48	33·40	37·38	34·12
Per cent of Expected	79·65	64·00	69·80	62·18
X-rays with local surgery.				
Number of cases of known duration	*21*	*9*	*9*	*19*
Mean number of months lived in 5 years from onset — Maximum possible	58·86	60·00	60·00	60·00
Mean number of months lived in 5 years from onset — Expected	55·38	55·61	53·77	54·80
Mean number of months lived in 5 years from onset — Actual	49·52	48·33	39·33	42·00
Per cent of Expected	89·42	86·91	73·14	76·64

The general conclusions to be drawn from the figures given above are :

1. The 5-year expectation of life after radical mastectomy ranges from 91·03 per cent of normal in stage I down to 68·4 per cent of normal in stage III*b*.

2. The 5-year expectation of life is not increased by post-operative radiation in stages I, II and III*a*, but in stage III*b* (78 cases) there was an increase of 4·3 per cent.

3. The number of patients treated by radical mastectomy with pre-operative radiation or implantation of radium at the time of operation was too small to admit of reliable conclusions being drawn from the figures.

4. Local mastectomy was the method of treatment in 17·0 per cent of those treated by operation, and, though the actual period of survival was much the same as after radical mastectomy, the 5-year expectation of life was relatively higher on account of the high mean age of these patients. These results were slightly improved by post-operative radiation.

5. Local excision of the tumour followed by interstitial radium or X-rays was the method used in a few cases, and gave a 5-year expectation of life almost equal to that afforded by radical mastectomy in stage I, and better than the latter in patients who were in the later stages, especially in stage III*b*, but the numbers so treated were small.

6. Radiotherapy alone gave a lower expectation of life.

7. It was found that patients in stage IV who were not treated either by surgery or radiotherapy had a 5-year expectation of life of 46·7 per cent of normal, which was not improved by palliative X-ray treatment.

All these figures must be considered in the light of the fact that the mean duration of the disease is 38·3 months (Major Greenwood, 1926), so that these patients whose mean age was 57 years would have a 5-year expectation of life of about 56 months ; a 5-year follow-up is therefore insufficient for assessment of the results of treatment. The variations in mean age of the patients submitted to various types of treatment is shown below, and indicates that the more radical methods were used in the younger patients.

	Years.
Mean age of 703 patients treated by radical mastectomy alone . .	54·11
,, ,, 393 ,, ,, ,, ,, combined with radiotherapy	51·18
Mean age of 133 patients treated by local mastectomy alone . .	63·44
,, ,, 100 ,, ,, ,, ,, combined with radiotherapy	59·50
Mean age of 174 patients treated by radium alone or with local surgery	58·25
,, ,, 393 ,, ,, H.V. X-rays alone or with radium and/or local surgery	60·46
Mean age of 232 patients not treated by surgery or radiotherapy . .	64·16

Pathological Report.

	Number.	Per cent.
No histological examination before or after death	743	35·0
Histological examination done before or after death	1386	65·0
Result doubtful	1	
The specimen examined was non-malignant	7	
Carcinoma, type unspecified	106	
Spheroidal cell carcinoma	980	
Adenocarcinoma	190	
,, colloid	28	
,, papillary	6	
Squamous cell carcinoma, keratinizing	2	
,, ,, non-keratinizing	2	
Sarcoma (type not specified 3, spindle cell 2, fibro-sarcoma 2, reticulum cell 1)	8	
Paget's nipple	3	
,, ,, with spheroidal cell carcinoma	7	
,, ,, ,, adenocarcinoma	1	
Duct carcinoma	45	

Histological Grade (according to Broders' classification).

Figures for this are omitted owing to the small proportion of cases graded.

Histological report on lymph nodes.

	Number.
No nodes found involved on histological examination	359
Axillary nodes ,, ,, ,,	625
Apical axillary (subclavicular) nodes found involved	36
Nodes not examined histologically	402

The percentage of cases in which there is disagreement between the clinical and histological findings is shown by the following figures:

984 *cases in which a histological examination was made.*

	Number.	Per cent.
No nodes involved clinically; confirmed on histological examination	252	61·3
No nodes involved clinically, but found involved on histological examination	159	38·7
	411	100·0
Nodes involved clinically, confirmed on histological examination	466	81·3
Nodes involved clinically, but found not involved on histological examination	107	18·7
	573	100·0

38·7 per cent more patients had the nodes involved than was evident clinically, whilst in those with nodes clinically involved the enlargement proved not to be malignant in 18·7 per cent.

Basis of diagnosis in 1018 *cases.*

Clinically malignant :	Number.	Per cent.
Confirmed by histological examination and/or autopsy	677	66·5
,, appearance of metastases or recurrence	201	19·7
Diagnosis based on clinical evidence only	123	12·1
Clinically benign :		
Proved malignant by histological examination	15	1·5
,, ,, appearance of metastases or recurrence	2	0·2

Other Primary Growths.

There were 60 patients who had growths in both breasts. In 54 patients the growth in the opposite breast was obviously a metastasis from the primary growth, but there were 6 patients in whose cases the second growth appeared to have started almost simultaneously in both breasts. There were also 23 patients who had had the opposite breast amputated for carcinoma from 1½ to 30 years previously. The average interval between the two growths was less than 5 years in 9 cases, 5–10 years in 8 cases, over 10 years in 5 cases, and unknown in 1. The mean of the intervals was 8·0 years.

Five patients had had cancer of some other organ for which they had been successfully treated ; 11 had simultaneous growths in other organs—bladder 1, uterus 2, lung 2, rodent ulcer 2, stomach 1, vulva 1, rectum 1, ovary 1 ; and there were 4 patients in whom second primaries appeared after the breast growths had been removed—rectum 2, cervix 1, rodent ulcer 1. None of these patients survived.

Cause of Death in 1382 *Patients.*

	Number.
Cachexia	1042
Cardio-vascular disease	47
Cerebral metastases	21
Uraemia	6
Pulmonary complications	95
Sepsis	14
Surgical shock	7
Haemorrhage	3
Pulmonary embolism	16
Intercurrent disease or unknown cause	131

Fourteen of the sixteen deaths from pulmonary embolism were post-operative at a mean interval of 11·5 days; in 1 case the operation had been performed 18 months previously and in 1 case there had been no operation. The diagnosis

was confirmed by autopsy in 12 cases, in 4 of which thrombosis of the femoral, subclavian or saphena vein was found to be the source of the embolus.

Autopsy.

	Number.	Per cent.
No autopsy	1246	90·1
Autopsy done	136	9·9

Relevant autopsy findings (multiple in most cases).

	Number.
Local growth only	22
Extension to neighbouring parts	20
Metastases in supraclavicular lymph nodes	22
,, mediastinal lymph nodes	24
,, lungs, pleurae and thoracic organs	55
,, liver and abdominal organs	62
,, brain	11
,, skeletal system	32
Pulmonary complications	41
Abdominal ,,	7
No growth found (post-operative deaths)	11

RECURRENT CASES—FEMALES.

There were 374 recurrent cases. The type of recurrence, nature of treatment of the primary, and the intervals from operation to first recurrence are shown in the following table.

Treatment of primary.	Number.	Local recurrence.	Metastases.	Local and metastases.	Mean interval in months.
Surgery alone	292	45	—	—	28·1
		—	169	—	37·9
		—	—	78	33·1
Surgery combined with radiation	63	9	—	—	36·6
		—	30	—	23·7
		—	—	24	30·6
Radiotherapy alone	19	4	—	—	10·3
		—	6	—	34·6
		—	—	9	37·4
Totals	374	58	205	111	

The longest intervals were in 2 patients who were found to have metastases in the lung (confirmed histologically) 23 and 20 years respectively after radical mastectomy. There were also 2 patients who had local recurrence (not confirmed

histologicallv) 16 and 17 years respectively after radical mastectomy. It will be seen that metastases were four times as frequent as local recurrence after radical surgery, and occurred most commonly in the fourth year after operation. The number of patients who had had post-operative radiotherapy was too small for the differences in times of appearance of local recurrence and metastases to be statistically significant.

The patients with local recurrence had a 5-year survival rate of 12·1 per cent. In the other two groups palliative treatment was given in less than half the patients, and the 5-year survival rates were 3·9 and 2·7 per cent respectively.

PRIMARY CASES—MALES (23).

Age distribution.

Age distribution.	Number.	Age distribution.	Number.
45–49	2	65–69	5
50–54	1	70–74	1
55–59	4	75–79	2
60–64	7	80–	1

	Males.	Females.
Mean age	63·5 ± 1·7	57·0 ± 0·4
Standard deviation	8·4 ± 1·2	12·6 ± 0·2
Difference of means	6·5 ± 1·78	

The difference between the mean ages of males and females is statistically significant.

Family history of cancer.

Fifteen patients gave no family history of cancer, and the question was not answered for the remaining 8.

First symptom.

	Number.	Per cent.
Lump in breast	15	65·2
Pain in breast	1	4·3
"Eczema" or ulceration of skin of breast	5	21·7
Bloody discharge from nipple	1	4·3
No symptoms—tumour discovered during routine examination	1	4·3

Interval from first symptom to first consultation.

34·8 per cent of the patients consulted a doctor within 3 months of noticing the first symptom, 26·1 per cent between the third and sixth months, and in 39·1 per cent more than 6 months had elapsed.

Findings on examination.

Location of tumour :	Number.	Per cent.
Right breast	12	52·2
Left ,,	11	47·8
Upper and outer quadrant	5	21·7
Central, behind nipple	15	65·2
Lower and outer quadrant	2	8·7
Diffuse, too advanced to identify primary site	1	4·3
Size of tumour :		
Small—up to 4 sq. cm.	5	21·7
Medium—4–20 sq. cm.	11	47·8
Large—20–100 sq. cm.	7	30·4

Clinical groups.

Stage I :	Number.
Confined to breast; lymph nodes not involved clinically	1
,, ,, ,, ,, histologically	1
	2
Stage II :	
Axillary lymph nodes involved clinically	4
,, ,, ,, histologically	2
	6
Stage III*a* :	
Fixed to skin or pectoral fascia, no nodes clinically	4
Stage III*b* :	
Fixed to skin or pectoral fascia, with nodes clinically	3
,, ,, ,, ,, histologically	4
	11
Stage IV:	
Supraclavicular lymph nodes involved	2
Remote metastases present	2
	4

The percentage of patients who were still in Stage I was only 8·7.

Methods of treatment and 5-year results.

	Number.	Operation fatalities.	Survived 5 years.	Died with cancer.	Died without cancer.	Not traced.	Operation fatalities. Per cent.
Radical mastectomy	8	1	2	1	3	1	12·5
Radical mastectomy followed by H.V. X-rays	2	—	1	1	—	—	—
Local mastectomy	3	—	1	2	—	—	—
Local mastectomy followed by H.V. X-rays	3	—	—	2	—	1	—
H.V. X-rays alone	1	—	—	1	—	—	—
H.V. X-rays followed by interstitial radium	2	—	—	2	—	—	—
Not treated	4	—	—	3	—	1	—
Totals	23	1	4	12	3	3	

Five-year follow-up results for all cases.

	First year.	Second year.	Third year.	Fourth year.	Fifth year.	Totals	
Operation fatalities	1	—	—	—	—	1	
Died with cancer	8	2	2	—	—	12	16
„ without cancer	—	2	—	—	1	3	
Alive and well	..	..	..	..	3		23
„ with cancer	..	..	..	..	1	4	
Untraced	..	..	..	..	3	3	
Total died yearly	9	4	2	—	1		

The 5-year survival rate for all cases was 17·4 per cent.

Five-year survival rate according to stages.

Stage.	Number.	Known survivors.	Per cent.
I	2	—	—
II	6	2	33·3
III*a*	4	1	25·0
III*b*	8	1	12·5
IV	3	—	—

The numbers in each group are so small that a detailed analysis of survival rate by methods of treatment and stages would not be statistically significant. An actuarial estimation of 5-year expectation of life of 8 patients who had radical mastectomy (Stage I, 2; Stage II, 2; Stage III*a*, 1; Stage III*b*, 3) gave an actual mean survival period of 36 months, which was 72·48 per cent of normal. If 2 patients who had post-operative radiotherapy are included, the figures are 38·9 months and 75·31 per cent of normal expectation.

Pathological report.

	Number.	Per cent.
No histological examination before or after death	8	34·8
Histological examination done before or after death	15	65·2
Spheroidal cell carcinoma	10	
Adenocarcinoma	1	
Duct carcinoma	2	
Basal cell carcinoma	2	

Histological report on lymph nodes.

No nodes found involved on histological examination	2
Axillary nodes ,, ,, ,,	9
Nodes not examined histologically	4

In 2 of the 9 positive cases the nodes were not involved clinically.

Basis of diagnosis.

	Number.	Per cent.
Clinically malignant, confirmed by histological examination	15	65·2
Clinically malignant, confirmed by appearance of metastases or recurrence	4	17·4
Diagnosis based on clinical evidence only	4	17·4

Cause of death in 16 patients.

	Number.
Cachexia	8
Cardio-vascular disease	2
Pulmonary complications	4
,, embolism	1
Unknown	1

Autopsy.

Autopsy was performed on 1 of the 16 patients who died, and multiple metastases were found.

RECURRENT CASES—MALES.

There were 3 patients with recurrences following radical mastectomy in 2 cases, and local mastectomy in 1. All 3 had had post-operative radiotherapy, and were in the third year following operation. All had metastases and died within a few months.

SUMMARY.

1. A statistical analysis of 2529 cases of cancer of the breast. 2152 of these were primary cases, 2129 females and 23 males.

2. 22·1 per cent of the female patients were single women and 77·8 per cent were married or widowed. The mean age of the single women was 55·6 years and that of the married and widowed 57·4 years.

3. The percentage of childless marriages amongst 1658 married and widowed women was 16·1 as compared with 12·5 in published control series.

4. A lump in the breast was the first complaint in 77·4 per cent of the patients, pain either local or referred in 11·6 per cent, discharge from the nipple in 2·2 per cent, and symptoms due to metastases in 1·6 per cent. In 1·6 per cent the tumour was discovered by the doctor or during routine examination, having caused no symptoms.

5. 44·1 per cent of the patients consulted a doctor within 3 months of noticing the first symptom, 15·3 per cent during the next 3 months, and in 33·3 per cent the symptoms were of over 6 months' duration before advice was sought.

6. 85·6 per cent of those who consulted a doctor were referred to hospital at once, but 3·6 per cent were told that the condition was not serious, and 4·8 per cent were treated by palliative methods for more than 6 months.

7. On admission to hospital it was found that 59·7 per cent of the patients had clinical involvement of the axillary lymph nodes and 10·3 per cent had clinically recognizable distant metastases. Four stages were defined, and in the final grouping 22·8 per cent of the patients were placed in Stage I, 21·9 per cent in Stage II, 35·7 per cent in Stage III and 17·1 per cent in Stage IV.

8. 703 patients were treated by radical mastectomy alone with an average 5-year survival rate of 47·6 per cent of traced cases, ranging from 68·2 per cent of those in Stage I down to 25·6 per cent in Stage III*b*; the operation mortality was 3·1 per cent.

393 patients were treated by radical mastectomy combined with radiotherapy, with an average 5-year survival rate of 39·9 per cent of traced cases, ranging from 64·1 per cent of those in Stage I, down to 30·1 per cent of those in Stage III*b*. Local mastectomy alone in 133 patients gave a 5-year survival rate of 59·6 per cent of traced cases in Stage I, down to 19·0 per cent in Stage III*b*; these results were improved when local mastectomy was supplemented by radiotherapy in 100 cases. The patients treated by local mastectomy were on the average older than those treated by radical mastectomy.

9. Treatment by radium and X-rays was usually only palliative, but there was a group of 45 cases in whom local excision of the tumour was followed by implantation of radium, with a survival rate of 63·4 per cent of traced cases for all stages.

10. The results of different methods of treatment were assessed by actuarial methods, in which allowance is made for the ages and consequent expectation of life of the patients. It was found that radical mastectomy gave a 5-year expectation of life ranging from 91·03 per cent of normal in Stage I, down to 68·4 per cent of normal in Stage III*b*; post-operative radiation improved these results by 4·3 per cent in Stage III*b*, in other stages the results were not so good as those of radical mastectomy alone. Other methods of treatment were assessed in the same way.

11. 31·2 per cent of all patients survived 5 years, ranging from 56·5 per cent of those in Stage I, down to 19·0 per cent of those in Stage III*b*, and 2·2 per cent of those in Stage IV.

12. Histological examinations were made in 65·0 per cent of the patients; 38·7 per cent who had no clinical evidence of involvement of the axillary lymph nodes were found on microscopical examination to have them involved; on the other hand, nodes which were considered on clinical grounds to be malignant were found in 18·7 per cent not to be so on microscopical examination.

13. There were 20 male patients, whose mean age was 63·5 years. Their 5-year survival rate was 17·4 per cent.

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THE DUODENAL SPREAD OF PYLORIC CARCINOMA.

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IN his 'Textbook of Pathological Anatomy' Rokitansky (1861) made his classical statement that pyloric cancer was exactly bounded by the pyloric ring, and that the growth never reached beyond into the duodenum. From this time it appears that the majority of observers, with the early exception of Brinton (1864), commented upon the integrity of the duodenum in cases of carcinoma of the stomach. Many well-known surgical teachers spoke of the habitual immunity of the duodenum from invasion. Kocher (1893), Mikulicz (1898) and Most (1899) believed it to be always constant. Kocher ventured that it was a problem of extreme interest to consider why gastric carcinoma grows in almost all cases towards the cardia, yet stops, on the contrary, at the duodeno-pyloric junction.

It was Brinton who first took especial exception to this proposition of Rokitansky. He brought against it criticisms founded upon numerous personal case-observations: " We may justifiably apply to it a criticism of unusual severity—a criticism which, even if it weigh every word, will scarcely do more than the author's (Rokitansky's) terse and weighty proposition really deserves." From 125 cancers of the pylorus studied by Brinton, there were no less than ten cases in which the disease was not bounded by the valve, but passed beyond it for a variable distance, often an inch or two inches, into the duodenum. He gave no information concerning the method of this spread. Brinton concluded by saying the rules which Rokitansky had the merit of laying down were, in this respect, like many others in pathology, of general though not of universal importance; their value was not much affected by occasional exceptions. This question of duodenal invasion by pyloric cancer seemed to present a surgical problem of

some magnitude, and the frequency and degree of its occurrence has been the object of several subsequent researches.

Carle and Fantino (1898), discussing the pathology and treatment of carcinoma of the stomach, expressed disagreement with Kocher. They mention three cases out of fourteen gastric resections in which neoplastic infiltration had spread under Brunner's glands for a distance of one to three centimetres. In addition, these authors stated that such a progression of the growth towards the duodenum necessitates an extension of the surgical resection as far as the second part of the duodenum.

Other later surgical authors, notably Borrmann (1901) and Cunéo (1903), attached considerable importance to Brinton's " occasional exceptions " with reference to the spread of cancer into the duodenum. Cunéo (1900), during his classical work on the lymphatic propagation of pyloric cancer, was already investigating the accepted state of immunity of the duodenum. He remarked on the disaccord between the macroscopic aspect—the abrupt termination of a gastric cancer at the level of the pylorus—and the results of the histological examination. This examination systematically diminished, he said, the percentage of the duodenal integrity.

Out of a large series of gastric carcinoma Cunéo (1903) took 11 cancers of the pylorus, and subjected their distal extension to a critical macroscopic and microscopic examination. He took his blocks of tissue from several places around the junction of the pylorus and duodenum. Before commenting upon his observations, he described very precisely his understanding of the surgical anatomy of this region. The thick pyloric muscle diminishes gradually towards the gastric side, but on the duodenal side the diminution is more abrupt, and the much thinner duodenal muscle begins immediately. Although the duodenal side of the pylorus is covered by duodenal mucosa, it must be considered surgically as part of the stomach. Therefore, Cunéo recognizes three regions : they are the gastric surface of the pylorus, the duodenal surface of the pylorus, and the duodenum proper.

On macroscopic examination of his 11 cases the duodenum seemed to be absolutely normal, but the duodenal surface of the pylorus appeared to be invaded by the growth in 8 of the cases. The neoplasm often resembled a big irregular cork protruding into the lumen of the duodenum, but surrounded by a " collar " of normal duodenum. On histological examination, however, four specimens showed that the neoplasm had infiltrated the first part of the duodenum. In three of these specimens (Cases 8, 12 and 15) it extended barely 5 mm. from the pylorus. In the other specimen (Case 16) there were submucosal and intramuscular malignant processes at about 1 cm. from the pylorus, and malignant disease was present right up to the line of resection. Cunéo felt that it was only this one case which could create any uneasiness, the infiltration being so close to the line usually chosen in gastric resection.

Borrmann's larger survey of 63 cases of pyloric cancer led him to far more pessimistic conclusions (Borrmann, 1901). Nineteen of his specimens showed duodenal invasion. In 9 of them the duodenum was diseased at the line of resection, even though this was at a distance of about 2 cm. from the pylorus. In what would concern the rough percentage of the duodenal invasion, Borrmann's figure was like that of Cunéo, namely about one-third, but the extent of the infiltration was much more considerable in Borrmann's cases. Cunéo,

familiar with Borrmann's findings, sums up from a surgical point of view. In many cases it must be necessary to view with suspicion the first 2 cm. of the duodenum. Logically the point of resection should be advanced up to 3 or even 4 cm. from the pylorus. But, he goes on to say, it is impossible to ignore the fact that such a wide resection complicates the operation, and necessitates a dissection of the duodenum from the pancreas.

The researches of Fenwick and Fenwick (1902) do not lend support to Brinton's findings, as they discovered out of a series of 87 pyloric cancers only 2 cases which demonstrated lymphatic spread into the duodenum. These authors remarked that though malignant growths of the pylorus may project into the lumen of the duodenum, they rarely implicated its walls. However, it is open to question whether their histological investigations were sufficiently careful to give reliable evidence, and the conclusions they advanced could scarcely have been founded upon their own results.

Moynihan (1926) was very familiar with the possibility of duodenal permeation in gastric cancer, and stressed that the duodenal integrity was more apparent than real. He quoted several of the authors already mentioned, and advocated the removal in all cases of pyloric cancer of the *whole* of the first portion of the duodenum.

Sherren (1932), describing the methods of spread of gastric cancer, mentioned his own surgical familiarity with duodenal infiltration by carcinoma. He gave no statistics of his own, but quoted those of Brinton, Borrmann and Fenwick.

It is interesting to consider the results reported in 1936 by Castleman from an examination of all the microscopical preparations of pyloric cancer from surgical and autopsy specimens, collected at the Massachusetts General Hospital in Boston during the period from 1900 to 1934. His findings can be divided into two groups:

(1) Out of 65 specimens from autopsies on pyloric cancer (the majority of which had been removed without the duodenum), in six cases only was it possible to identify an invasion of the duodenum. From 134 surgical specimens, 15 only showed an extension into the duodenum. Out of these 15 specimens, 9 revealed cancer cells up to the edge of the duodenal resection, indicating the probable persistence of cancerous elements in the remaining duodenum stump. In only 6 cases did there exist a band of normal duodenum beyond the extreme limit of the cancer mass.

(2) Towards the end of 1934 special attention was given to this subject, and care was taken to select blocks of tissue for section from the extreme margin of the resected specimens. Following the introduction of this new technique, the proportion of cases showing duodenal propagation of cancer during the course of one year was found to be much greater; 7 out of 28 pyloric cancers showed some extension into the duodenum. The distances of this extension varied from 3 mm. to 3 cm. The spread, which rarely reached the mucosa, extended in the submucosa below the glands of Brunner.

Several authors have published single detailed case-reports (Heiberg, 1937; Loewy and Bertrand, 1939; Cain and Claisse, 1939), in which unusual histological features have been stressed, such as an extension of the growth into the second part of the duodenum, and diffuse lymphatic permeation of the duodenal mucosal plexus. One item of additional interest in the case-report of Loewy and Bertrand is the post-operative X-ray therapy administered because of neoplastic infiltration

of the duodenum seen during gastrectomy. This is probably the only recorded instance of deep irradiation therapy given for this very specific purpose.

Ewing (1940) is brief upon this subject, and cites a single personal case observation where a carcinoma of the pylorus extended over the first 2 cm. of the duodenal mucosa.

Walters, Gray and Priestley (1942), publishing a comprehensive treatise upon the pathology and surgical therapy of 10,000 cases of gastric cancer in the Mayo Clinic, failed to mention microscopical duodenal spread of the growth. In spite of Castleman's findings the usual surgical statement is made without further qualification, namely: "Gross involvement of the duodenum by gastric cancer is rare . . . distal extension of the growth usually ends abruptly at the pylorus. For this reason it is most unusual to discover that a lesion is inoperable because of extension in this direction." Whilst admitting that the latter part of this statement may be very true, in several instances the patient might ultimately fail to share its benefits with the surgeon.

Willis (1948) condemns the notion of duodenal immunity from cancerous permeation. In a personal communication he mentions two incidental specimens of gastric carcinoma which clearly demonstrated invasion, and illustrates lymphatic spread of the growth within the submucosa and muscularis.

Anatomical.

In addition to the simple factual problem of duodenal invasion by gastric cancer, it is necessary to consider carefully at least two other related problems. One is the routes most favoured by cancer in its progress into and along the duodenal wall; the other is the old question asked by Brinton, Kocher and Cunéo, namely why gastric cancer should ever experience a visible check to its spread at the pyloro-duodenal junction. A study of the microscopical anatomy of this region, in relation to the morbid process *in situ*, may help to clear up some components of each problem.

To Cunéo (1900) the apparent arrest of a cancer at the pylorus was due, above all, to a mechanical cause. For him it depended upon an absence of continuity between the gastric and duodenal submucous spaces—a continuity broken by the annular muscle of the pylorus, and by a condensation of cellular tissue within the submucosa to form a dividing septum. His gelatine injections into the gastric submucosa near the pylorus met with an "invincible obstacle." At the same time he admitted that though it would be ludicrous to consider such barriers as absolute in opposing gastric cancer, they still played the principal role in the protection of the duodenum against neoplastic invasion.

For Brinton (1864) and for Fenwick and Fenwick (1902) the reason was again anatomical. The muscular coats of the two viscera along the line of fusion were so distinct from one another that a direct continuity could scarcely be said to exist. These authors made the analogy between the duodenal attachment to the stomach and the manner in which the vagina embraces the neck of the uterus. To them a growth of the pylorus was more apt to infiltrate the contiguous walls of the stomach than to extend obliquely along a comparatively thin and external layer of tissue into the wall of the gut.

More recently Horton (1928) made a very detailed study of 5000 sections from the stomachs of 84 subjects, in order to determine the percentage of pyloric

circular and longitudinal muscle fibres continuous with the corresponding fibres of the duodenum. Eighty-one of these specimens showed a complete discontinuity between the circular muscle of the pylorus and that of the duodenum. In all 84 specimens, about 21 to 24 per cent of the longitudinal fibres of the pylorus were continuous with the longitudinal muscle fibres of the duodenum. One subject did not show continuation of either the circular or longitudinal fibres from pylorus into duodenum.

With regard to the lymphatic communication between the two viscera, since Cunéo (1900) demonstrated so fully that the spread of gastric carcinoma was dependent in the main upon the lymphatics of the stomach wall, the relative infrequency of spread into the duodenum seemed to cast doubt upon any continuity between the gastric and duodenal lymphatic plexuses. Cunéo and Most (1899) maintained, however, that there was a communication between the lymphatics of the gastric submucosa and those of the duodenal submucosa.

By injection experiments, Jamieson and Dobson (1907) concluded that there was undoubtedly a direct continuity between the subserosal plexuses. Injection of the pyloric submucous plexus showed a spread of fluid into the corresponding duodenal plexus. Lymphatic vessels also carried the injection fluid through the pyloric muscle layer into large numbers of vessels on the upper and lower surfaces of the duodenum. This latter formed such a free indirect communication between the submucous and subserous plexuses that any question about a *direct* longitudinal continuity between the two submucous plexuses was considered to be of little importance. Their experiments made it possible for them to conclude that such a plentiful anastomosis existed between the lymphatic plexuses of the stomach and duodenum that the accepted comparative integrity of the duodenum was not easily explained by the disposition of the lymphatics. In their opinion it was obvious that the duodenum may possibly be invaded from the pylorus by the extension of cancer along the submucous and subserous channels. In addition, both Jamieson and Dobson and Cunéo emphasized the final possibility of retrograde lymphatic spread. Many collecting vessels from the pyloric subserous network run downwards over the duodenum to reach the subpyloric or retropyloric group of lymph nodes. As these nodes also receive vessels from the duodenum, an indirect communication also exists. That this may be of some importance was stressed by Moynihan (1926) when he says that the implication of a single gland may so sufficiently disturb the direction of the lymph current that cancer cells from a primary growth may pursue an erratic course.

Horton (1928) working upon stomachs removed from young subjects one to three hours after death, injected 35 with coloured gelatine or Indian ink. The injection needle was inserted in several places along the pyloric submucosa. This author disagrees with Jamieson and Dobson, and does not recognize any direct communication between the submucous lymphatics of the stomach and the duodenum, as most of his preparations showed only subserous lymphatic continuity.

OBSERVATIONS.

Over a period of nine months all surgical and autopsy specimens of pyloric cancer received by the Department of Pathology of the University of Liverpool, and those submitted to the Liverpool Cancer Control Organization by the Pathologists of the Liverpool Regional Hospitals, were examined macroscopically and

microscopically specifically for evidence of such a spread of the growth. The total number of specimens so examined was 50. Twenty-one were from autopsies and 29 were from surgical cases.

From each specimen several blocks of tissue were taken—from the distal extremity of the growth, from the pyloro-duodenal junction, and from the adjoining duodenum. In the surgical group the total length of the resected duodenum, ranging in length from 1·0 to 2·8 cm. in the fixed specimen, was included for section. The whole of the first part of the duodenum was included in the blocks from autopsy specimens; greater lengths were studied in several instances where macroscopic examination indicated the need for this. Following Borrmann's segregation of the tumour spread to those walls of the duodenum which correspond to the gastric greater and lesser curvatures, blocks from every case included tissue along the lines of these curvatures.

Out of these 50 cases 16 demonstrated a spread of the tumour into the duodenum, 9 from the surgical and 7 from the autopsy group. In the surgical cases the growth had spread into the duodenum for distances varying from 0·5 to 2·5 cm., and in 5 cases collections of cancer cells had reached the edge of the resection (Fig. 1). It does not seem necessary for the primary pyloric growth to be large before it involves the duodenal wall. Two of the present surgical specimens were small cancer-ulcers upon the lesser curvature of the pyloric antrum, and cancer had invaded on the gastric side only the submucosa, with extension in one case into the pyloric sphincter muscle. Yet both these specimens showed cancer islands within duodenal lymphatics at a distance of almost 1·5 cm. from the pyloro-duodenal junction. This was also instanced by a Cabot Case No. 25472 (1939) of the Massachusetts General Hospital, in which specimen a small malignant ulcer of the pylorus was limited to the mucosa except for a small extension below the muscularis mucosae. Around the ulcer cancerous infiltration had spread for a considerable distance under the normal mucosal glands, and had passed the pylorus for several millimetres into the duodenal submucosa.

From this study it appears that the infiltration takes place far more frequently along the duodenal continuation of the lesser curvature than along that of the greater curvature. Spread along the latter was frequently seen to stop immediately after the pylorus had been passed, and could not be picked up again

EXPLANATION OF PLATES.

FIG. 1.—Lymphatic cancer in the submucosa and muscularis of the duodenum. The irregular edge of the section is the resection edge of the surgical specimen. × 34.

FIG. 2.—Surgical specimen showing a solitary focus of mucoid carcinoma in a lymphatic within the Brunner gland layer, approximately 1·5 cm. distant from the pylorus. × 200.

FIG. 3.—Intra-lymphatic cancer traversing the pyloric sphincter muscle to reach the duodenal longitudinal muscle (bottom right hand). Cancer is also beginning to infiltrate the duodenal submucosa. × 30.

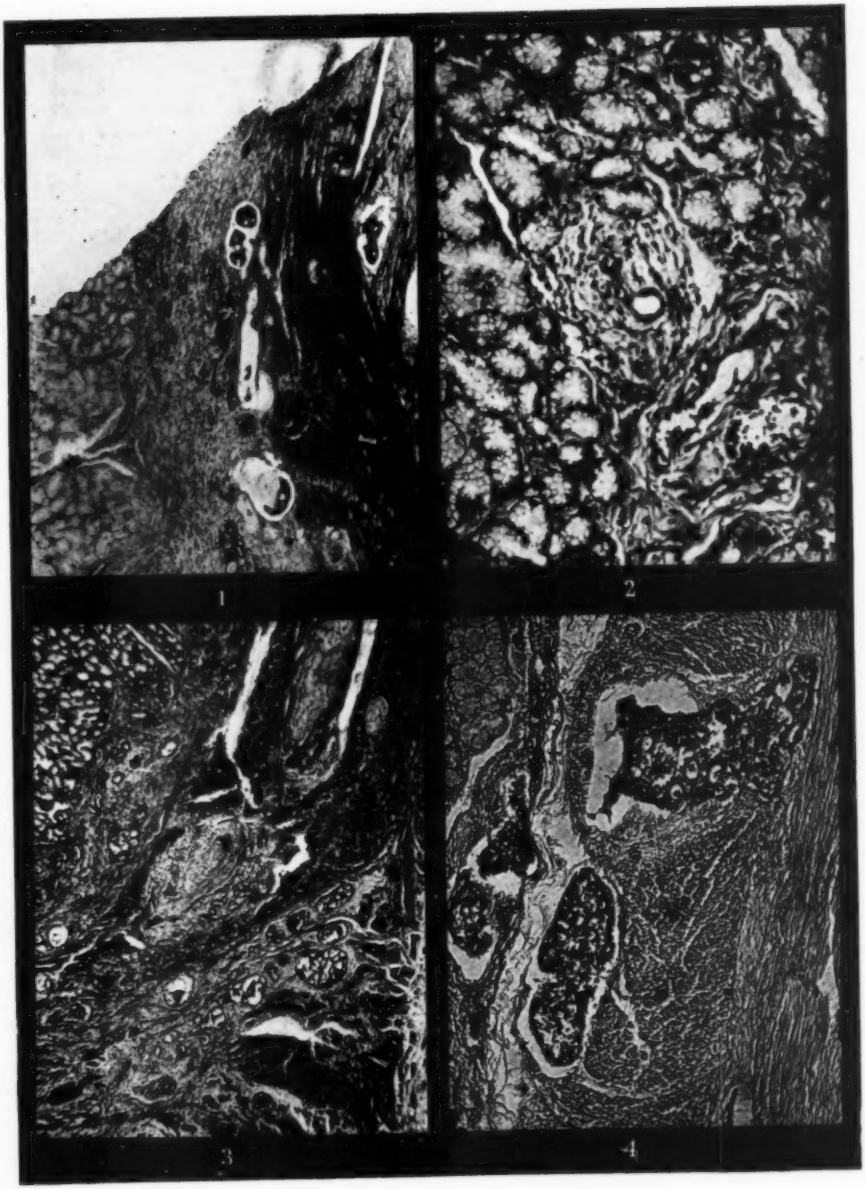
FIG. 4.—Cancer masses permeating lymphatic channels through the duodenal muscle to reach the mucosal plexuses above. × 60.

FIG. 5.—Lymphatic permeation through the Brunner gland layer. × 62.

FIG. 6.—Same as Fig. 7. × 48.

FIG. 7.—Direct contiguous submucosal spread from the pylorus. × 56.

FIG. 8.—Direct cancer spread from the pylorus with accompanying lymphatic permeation. Both the submucosal and circular muscle layers are being infiltrated by the growth. × 56.



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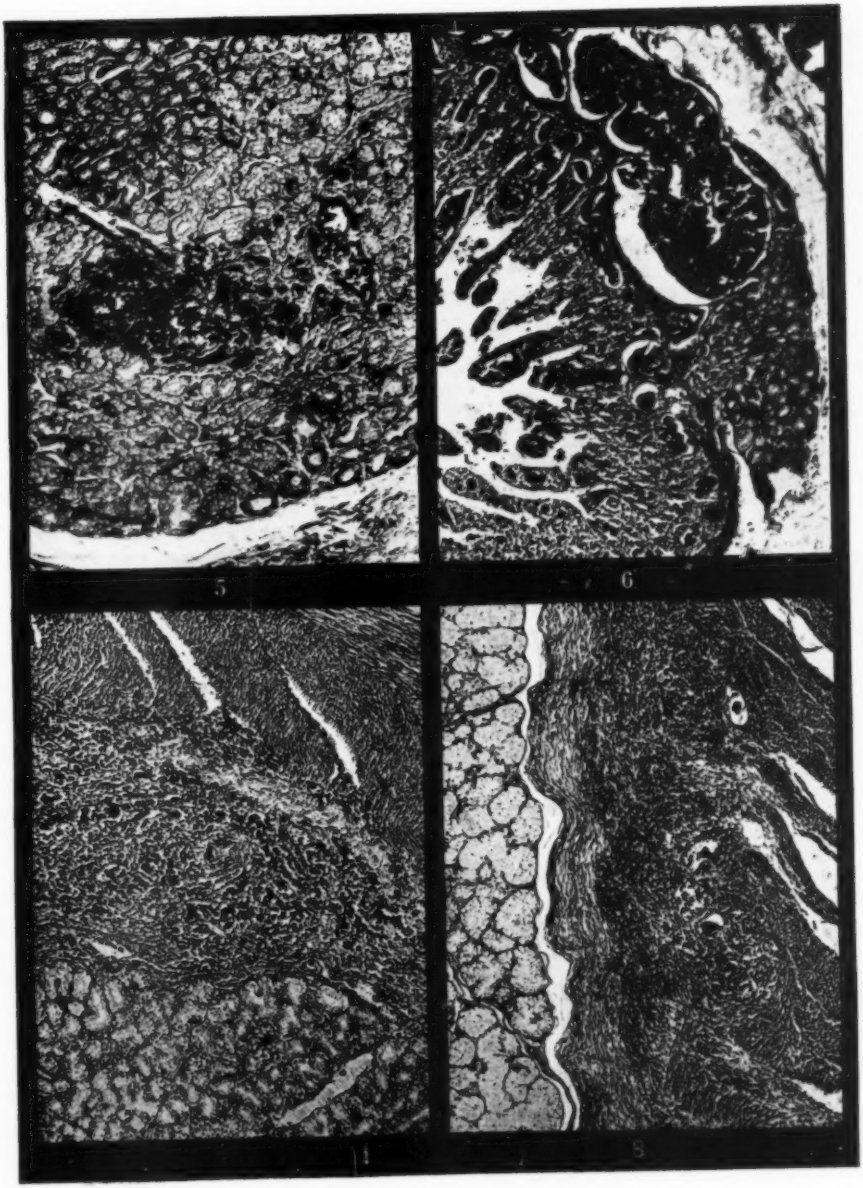

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in the sections, whereas in corresponding sections from the lesser curvature progression of the growth was often well distant from the pylorus.

From most of the sections it did appear that the bulk of the lymphatic cancer spread in the pyloric submucosa suffered some check at the sphincter, though not an absolute one. It was a common microscopical finding, especially in the surgical cases, to see beneath the carcinoma in the gastric mucosa the broad, normal-looking pyloric muscle, forming as it were, a muscular precipice down which there was no sign of cancer ; then at the foot of this steep to come abruptly upon a few lymphatic cancer islands, some or all of which were within the duodenal wall. Similarly, solitary nests of cancer cells were not infrequently discovered in almost any situation in the duodenal wall, distant by many millimetres from the pylorus, with no indication as to their route of arrival (Fig. 2). In other sections, however, it was possible to determine that many of the cancer groups from the gastric submucosa traversed lymphatic channels actually through the circular sphincter muscle of the pylorus, gained the outer muscular and the subserosal layers, and used tracts within these tissues to reach the longitudinal muscle and subserosa of the duodenum (Fig. 3). Once these positions had been attained these cell groups seemed to have no difficulty in their longitudinal progression. In addition, many of them spreading in the lymphatics within these external layers crossed vertically through the duodenal circular muscle (Fig. 4), some to insinuate themselves along the fine connective-tissue septa between the Brunner glands (Fig. 5), but the majority to turn distally once more, now within the submucosa. Permeation of the lymphatic channels within this latter layer was also seen to occur from lymphatic cancer masses within the pyloric muscle directly into the duodenal submucosa.

The autopsy series naturally showed a later stage of the spread of carcinoma into the duodenum. Subserosal and frequently submucosal cancer spread was macroscopically visible. The duodenal mucosa was often wrinkled and nodular, and no sharp margin could be seen between the mucous membrane of stomach and of duodenum. Microscopically there was an extensive lymphatic spread of the pyloric growth within all layers of the duodenal wall, but especially within the submucous and subserous network, and sometimes along both curvature lines. The circular and longitudinal muscle layers were frequently broken up by a disorderly cancerous infiltration. In two specimens cancer spread of this nature had reached to, and included, the ampullary portion of the duodenum. Masses of tumour tissue had infiltrated the thickened duodenal muscularis mucosae, and there was an extensive permeation of the periglandular and subglandular plexuses (Fig. 6). The central lymph vessels of many mucosal villi were filled with cancer, and in one case there was focal superficial mucosal ulceration, leaving exposed cancer masses within the subglandular plexus. Permeation of these plexuses in the surface mucosa was rarely seen, and then only in association with cancerous filling of the other larger plexuses.

One does not receive the impression microscopically that the pyloric " front " of the growth in the stomach is facing and being held up by any impenetrable duodenal barrier. The only structure apparently offering any kind of barrier action was the gland layer of Brunner. It has been the experience of the majority of authors that the layer of Brunner glands remains free, in spite of many, and long, extensions of gastric cancer beyond the pylorus. In addition, whatever the method of propagation, it is exceptional that the mucosal epithelium of the

duodenum itself is invaded, though no normal anatomical barrier interrupts the continuity of this surface epithelium between pylorus and duodenum. Sections from those blocks through the pyloro-duodenal junction showed that in 32 cases the mucosa of the duodenal surface of the pylorus had been replaced in part or totally by the pyloric growth, yet in no instance had this mucosal invasion extended directly along into the duodenum proper. The duodenal mucosa does not appear to constitute a favourable terrain for the spread of cancer, and the two cases of its involvement seen in this review were dependent upon the diffuse periglandular lymphatic permeation spreading upwards in a flanking manner from the larger plexuses beneath. It is highly probable that the few instances of direct mucosal invasion, reported and described as a rare feature by some authors, are in actual fact manifestations of this indirect mucosal permeation. Every detailed case-report of so-called direct mucosal spread has a description of cancerous masses within the underlying lymphatic plexuses.

At the level of the pyloric orifice there exists a pronounced dissociation between the progression of a tumour in the connective-tissue spaces and the progression by the lymphatic pathways. This dissociation is far from being absolute, and there is, though not commonly, direct contiguous growth of cancer into the duodenum, between longitudinal muscle fibres and within the tissues of the submucosa and serosa. Out of the 50 cases in this review, it was well demonstrated in 4; the growth coming directly onwards from the sphincter muscle (Figs. 7 and 8). There was always an accompanying lymphatic spread, but in two of these cases the direct spread appeared in advance of it. Borrmann had two similar cases which showed this continued distal growth for more than 2 cm.

Observations upon the state of lymph nodes and neighbouring viscera, relative to the distal spread of pyloric cancer will be described in a subsequent paper.

DISCUSSION.

It must be the experience of every surgeon and pathologist that the vast majority of pyloric cancers do apparently end abruptly at the beginning of the duodenum. This cannot be said with the same affirmation after these cancers have been subjected to careful histological scrutiny. That a big discrepancy does exist is now more than a supposition, and the idea of the integrity of the duodenum in pyloric cancer—a notion which is still accepted in spite of contrary researches of several investigators—is only slowly receiving the necessary revision. Anatomical pathways exist in plenty between the two viscera, proved both by experiment and the natural migration of cancer cells (Fig. 9). Whether these lymphatic plexuses communicate more freely in the submucosa or in the subserosa, it matters little; cancer cells are furnished with, and make use of, such prepared pathways for overcoming any pyloric obstacle and invading the duodenum. One feels that the possible explanation of this apparent macroscopical check to the growth lies to a large extent with the mucosal and Brunner gland layers. It has been seen that these duodenal tissues remain very averse to the spread of the neoplasm, and such a resistance may be related to the rarity of primary cancer at this site.

To give adequate surgical emphasis to this discrepancy Castleman's (1936) results alone need only be realized in a manner such as the following: From

100 cases of pyloric cancer it would be likely that 25 of them would show duodenal invasion microscopically; and in 15 out of these 25 cases the surgical resection of about 1·5 cm. to 2 cm. of duodenum would have been insufficient. Ideally an obvious check upon the surgical part of this problem would be the examination of the remaining duodenal stump in patients who die some months or years after gastrectomy for pyloric cancer. In addition similar examination of those cases which constitute an early operative mortality for every surgeon could be undertaken. One case (B.363) from the autopsy group of the Liverpool Cancer Control collection would appear to provide an interesting reflection upon this point. A gastrectomy was performed in 1940 for adenocarcinoma of the pyloric antrum. Approximately five years later the patient died in hospital, and at post-mortem there was, in addition to secondary growth of cancer in the para-aortic lymph nodes, what appeared to be a large primary cancer of the second part of the

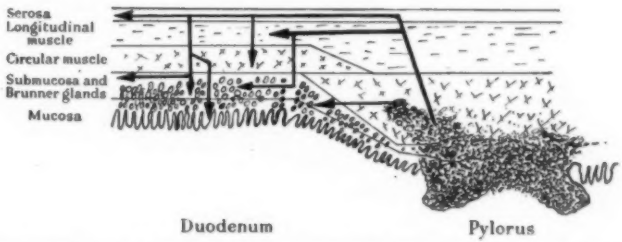

FIG. 9.—Diagram of the main pathways of cancer spread from the pylorus into the tissues of the duodenum.

duodenum. The histology report at that time was that this tumour, an adenocarcinoma, was arising, not from the bile ducts or ampulla, but from the duodenal mucosa. In all the layers, lymphatics throughout the entire length of the duodenal section were distended with neoplastic columnar epithelium.

In all the surgical cases except one the duodenal extension of the growth was microscopic. There was no single macroscopic feature which could offer to the surgeon a guide as to whether or not such infiltration had taken place. The size of the primary growth should by no means be relied upon, for a small growth can give rise to an extensive duodenal lymphatic permeation. In the single instance of macroscopic spread, a perfectly mobile pylorus showed at operation visible subserous duodenal infiltration. A resection of almost 3 cm. of the duodenum was made. Microscopically the duodenal growth was so proliferative in the muscular and serous layers that one could not avoid an impression that an even greater length of duodenal resection would have been advisable. Though several authors advocate the routine adoption of a longer duodenal resection, they rarely mention the appreciable increase in the technical difficulties involved. A rise in operative mortality would be almost inevitable, and would possibly outweigh any advantages. Possibly the problem could be met in the future by the combined effect of surgery and post-gastrectomy irradiation therapy, the latter administered through the laparotomy incision in a manner like that advocated by Fairchild and Shorter (1947).

TABLE I.—*The Relationship of Type of Tumour to its Duodenal Spread.*

		Total.	Number positive.	Number beyond normal line of resection.
Surgical specimens	Scirrhous	17	7	4
	Encephaloid	12	2	1
Autopsy specimens	Scirrhous	13	5	2
	Encephaloid	8	2	—

Finally, considering the results shown in Table I, it is interesting to note the views of Stewart (1947) upon the prognosis of gastric cancer after radical operation. If left without operation the encephaloid type kills more quickly than the scirrhous. But if an apparently adequate gastrectomy is done as soon as the condition is diagnosed the encephaloid gives an incomparably better prognosis than the scirrhous type. This is probably not only a matter of earlier diagnosis of encephaloid tumours; there appears to be a very early lymphatic permeation of a scirrhous carcinoma beyond the confines of the stomach and the regional lymph nodes.

SUMMARY.

Fifty cases of pyloric carcinoma were investigated with a view to determining the frequency and the methods of spread of the neoplasm into the first part of the duodenum. Sixteen cases showed that such an invasion of the duodenum had occurred, the invasion being more widespread and destructive in autopsy cases than in surgical cases. Nevertheless, in five out of the nine surgical specimens showing such duodenal involvement, cancer cell groups were seen at the edge of the duodenal resection. Progression of the growth occurred mainly through the lymphatics of the duodenal wall, and demonstrated an anastomosis between the pyloric lymphatic plexuses and those of the first part of the duodenum. In a smaller proportion of cases the pyloric neoplasm invaded the duodenal tissues directly. It was felt that the familiar macroscopic check to pyloric cancers at the duodenum was due to the duodenal mucous membrane and the layer of Brunner glands. Microscopically these were the only tissues which appeared to resist the spread and destructive effects of the growth.

ACKNOWLEDGMENTS.

I would like to express my gratitude to Professor H. L. Sheehan for valuable criticisms and suggestions throughout this work, Professor T. B. Davie for his help with the plan of this research during its initial stages, and those Pathologists and Surgeons of the Liverpool Regional Hospitals who have so kindly supplied me with specimens and helpful data. My thanks are due to Mr. D. Taylor and Miss M. Litchfield for all my numerous histological preparations, and to Mr. F. Beckwith, F.I.M.L.T., for my photomicrographs.

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SQUAMOUS CELL CARCINOMA OCCURRING IN ASBESTOSIS OF THE LUNG.

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RECENT reviews of the literature on the association between asbestosis and lung cancer reveal different opinions as to whether asbestosis has been an aetiological factor in these cases.

Homburger, in 1943, reviewing nineteen known cases found no reliable answer to the problem, whereas Wedler in the same year considered there was a significantly high incidence of lung cancers in autopsied cases of asbestosis. Kennaway and Kennaway (1947), who examined this problem as part of their broader statistical study of lung cancer, also leave the question unanswered. The statistical difficulties are due to the small numbers of asbestos workers, and to paucity of published autopsy records of such cases.

The following case is therefore presented :

Clinical history.

A female, aged 37, married, was admitted on August 27, 1945, to the Royal Victoria Infirmary, Newcastle-upon-Tyne. She complained of cough, haemoptyses and loss of weight for about four months, but since October, 1944, she had been feeling listless and noticing palpitations and dyspnoea on exertion. While in hospital she developed a small left-sided pleural effusion, and clinical signs suggested a diagnosis of pulmonary neoplasm. She began to develop paroxysms of breathlessness, occurring two or three times a day, and in one of these she died on September 17, 1945.

Past history.—Worked in a factory making asbestos pipe covers from age 15 to 22 years. The patient's aunt had worked in the same factory, and she died of asbestosis at the age of 40.

Autopsy findings.

Autopsy was performed on September 18, 1945. The body was that of a normally developed but poorly nourished middle-aged female subject. Skin showed numerous striae atrophicae; the mucous membranes were pale, and there was slight subcutaneous oedema at the ankles.

Pleurae.—Left: obliterated by fibrous adhesions except over upper part of upper lobe, where a small blood-stained exudate was present. Right: a little recent fibrinous pleurisy over lower lobe and 100 c.c. of fibrinous yellow fluid.

Pericardium.—Obliterated by fibrous adhesions, and infiltrated by nodular masses of friable neoplastic tissue.

Peritoneum.—Generally smooth and shining. Contained 200 c.c. opalescent yellow fluid with slight fibrin content. Greater omentum showed a solitary nodule of necrotic tumour tissue, 2·5 cm. in diameter.

Lungs and heart.—(Unweighed.) Were fixed in formalin and sectioned at 1·5 cm. intervals in a coronal plane.

Lungs.—Situated 5 cm. from the carina, in the bronchus to the left lower lobe, there was a neoplasm producing virtually complete obstruction to the lumen of that bronchus. The neoplasm had spread locally, invading the left lower lobe and the adjacent broncho-pulmonary and tracheo-bronchial lymph nodes, forming a mass of tumour tissue measuring approximately 7 × 10 × 6 cm. The cut surface showed that most of the tumour was composed of firm, white friable tissue, but frequent small areas of yellowish soft necrotic tumour tissue were present. Incorporated remains of anthracotic lymph nodes were also seen. A little lymphatic permeation was traced to the base of this lobe and involved the diaphragm. The lower part of the lower lobe showed a fairly marked collapse together with a slight fibrosis.

The left upper lobe was well aërated, but showed a sharply defined, firm area of congestion (suggesting venous infarction). The pulmonary artery to this

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EXPLANATION OF PLATES.

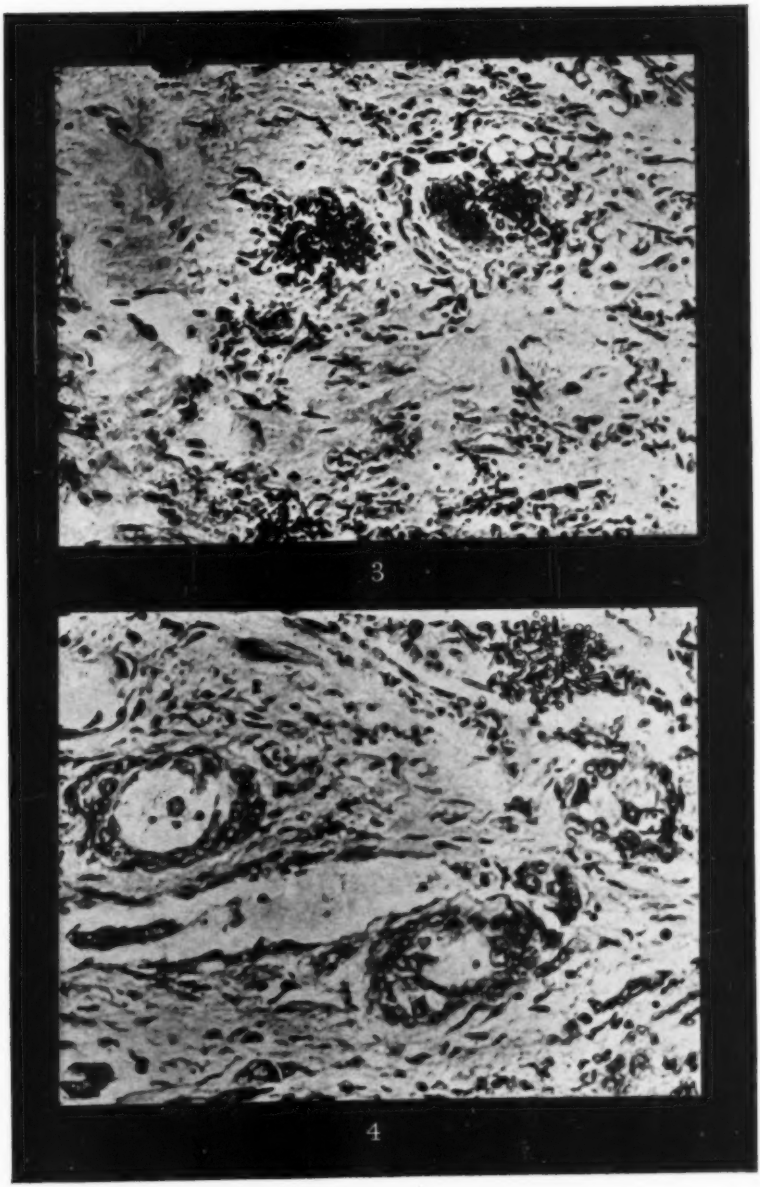
FIG. 1.—Slice of lung showing the neoplastic mass and the fibrosis at the lung bases.

FIG. 2.—Asbestos bodies and phagocytes lying in the alveoli. H. and E. × 480.

FIG. 3.—Extensive fibrosis in the lung. Masses of asbestos bodies and foreign body giant cell reaction. H. and E. × 100.

FIG. 4.—Adenocarcinomatous type of neoplasm with mucus secretion. H. and E. × 150.

Cureton.



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4

Cureton.

lobe showed slight stenosis, and the vein contained some ante-mortem thrombus. This had resulted from neoplastic invasion of the vein penetrating the intima. The related broncho-pulmonary lymph nodes were unaffected. The right lung was well aërated, showed no neoplastic infiltration or infection, but there was slight basal collapse and fibrosis as in the left lung. The left dome of the diaphragm showed scarring and was firmly united to the lung.

Heart and mediastinum.—Anteriorly the neoplasm had completely surrounded the aortic arch and the main pulmonary vessels, which showed some degree of stenosis. The pericardium had been infiltrated by nodular neoplastic growth penetrating into the myocardium of all the chambers of the heart, but not reaching the endocardium. Posteriorly the growth extended to the wall of the oesophagus, but no actual infiltration of this structure or of the superior vena cava was found.

Liver.—Weight, 1480 g. Showed a rather small number of metastases, (the largest being 0·6 cm. in diameter) and a cavernous haemangioma measuring 0·6 cm. in diameter.

Kidneys.—Right, 150 g.; Left, 160 g. Showed occasional metastases, 0·2 cm. in diameter, but no other significant change.

Ovaries.—Left (2·5 × 1·5 × 1·5 cm.) showed corpora lutea of varying age but normal appearances. Right (3 × 2·5 × 2·5 cm.) contained a nodule of partially necrotic tumour tissue, 2 × 1·5 cm.

Right femur.—Showed multiple lines of arrested growth. Other organs and bones showed no significant pathological change.

Histology.

Lungs: Section through the stenosed point of the left lower lobe of the bronchus shows a non-keratinizing squamous cell carinoma lining the lumen and infiltrating through the whole wall of the bronchus, but without invading cartilage. The mucous glands are distended and occasionally themselves infiltrated. There is some lymphatic permeation. The pulmonary artery in this section shows no infiltration. Above the level of the neoplasm the bronchus is lined by normal ciliated columnar epithelium. No squamous metaplasia is present. Tumour necroses and mitotic figures are very frequent. Adjacent broncho-pulmonary lymph nodes are almost completely infiltrated.

Section of a main pulmonary vein shows neoplastic cells infiltrating the wall of the vessel; in places these have reached the lumen and lined the wall with a thin layer of squamous carcinoma. Ante-mortem thrombi have resulted, and some regions show early organization of the thrombus, which itself contains typical neoplastic cells.

Section of the left lower lobe shows lymphatic permeation by neoplasm, marked collapse and areas of oedema and haemorrhage. Intra-alveolar phagocytes containing iron and carbon are numerous, and foreign body giant cells are seen in relation to the typical golden-yellow asbestos bodies lying in the alveoli and the finer lung trabeculae. There is marked subpleural fibrosis not related to neoplastic infiltration, but a consequence of the asbestosis. The bronchioles are closely set and their muscle and elastic coats are largely destroyed. One section of this lobe shows that the neoplastic cells, although preserving most of the features of squamous carcinoma, nevertheless show a definite tendency to acinus formation, and here there is much secretion of mucus into the intercellular

spaces, and numbers of the tumour cells have become distended by mucous vacuoles.

The left upper lobe shows similar asbestos bodies, but no fibrosis is seen. The picture is one of emphysema, gross veno-capillary congestion with extravasation of red cells into the alveoli. This corresponds to the area described as a venous infection.

Sections of the right lung confirm the presence of asbestos bodies, lower lobe fibrosis, and absence of any neoplastic tissue.

Heart : Section shows neoplastic infiltration of pericardium and outer part of the myocardium.

Liver shows two small very early metastases, and appearances of a chronic venous congestion with fatty change.

Kidneys : Sections show occasional very early metastases as in previous section.

Diaphragm shows extensive scarring and neoplastic infiltration.

Omentum shows a neoplastic nodule, part of which is necrotic and shows some superimposed inflammatory reaction.

Right ovary : Similar neoplastic infiltration is seen.

Spleen, Thyroid, Suprarenal, Ileum : No significant change.

COMMENTARY.

Careful search was made of the hospital autopsy records since the histological appearances of asbestosis were first described by Professor MacDonald in 1927, but no other cases of asbestosis were found.

Literature regarding the association between asbestosis and the development of neoplasms leaves the question still open.

Homburger (1943), reviewing 19 known cases up to 1943, finds that neither from statistical calculations nor from purely morphological studies is there any reliable answer to the question. Wedler (1943) reviews 14 cases up to 1943 out of 92 post-mortems on asbestosis cases (16 per cent), and considers this is a significant increase above the proportion of lung cancer in autopsies generally (2 to 6 per cent). The Senior Medical Inspector of Factories (1939) has reported 12 cases of cancer of the lung occurring in 103 fatal cases of asbestosis (11·6 per cent). Kennaway and Kennaway (1947) examined the death certificates of cancer of the lung in males between 1921 and 1938, and found 8 such cases (occurring in males between the ages of 45 and 64). In the general population there was an incidence of 8 carcinomata of the lung in 4000 males of the same age group. They were unable to acquire any exact figure regarding the number of persons exposed to the hazard in this country, and therefore make no conclusions.

This case showed a long latent period of fifteen to twenty-two years between exposure to the asbestos hazard and the development of a neoplasm—a fact noted previously (Lynch and Smith, 1935 ; Gloyne, 1935 ; Egbert and Geiger, 1936). The period of exposure also was long (seven years), and although this is usual, cases have been reported of a neoplasm developing after considerably shorter exposures. The age of the patient in this case is much younger than those tabulated by Kennaway and Kennaway, and would appear definitely on the low side for a squamous growth, which usually affects a rather higher age-group than the oat-cell type.

The neoplasm, although mainly a typical squamous type, showed a definite tendency to gland-like arrangement associated with much extracellular mucus. Variation in cell type is, of course, a recognized feature of many bronchogenic growths. Attention has frequently been drawn to the presence of squamous metaplasia in lung fibrosis and its possible relation to a squamous carcinoma. In this case no evidence of squamous metaplasia was found, although many blocks of tissue were examined. Homburger found only one instance of metaplasia occurring in pneumoconioses, and considers it a very doubtful indication that asbestosis predisposes to carcinoma. The literature is fully reviewed in his article.

I should like to express my grateful thanks to Professor Geoffrey Hadfield and to Professor Sir Ernest Kennaway for their criticisms and great help, and also to Messrs. A. Harvey and A. Young of the Department of Pathology, Royal Victoria Infirmary, for invaluable technical assistance.

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HAEMAGGLUTINATION BY EXTRACTS OF TUMOURS AND OF SOME NORMAL TISSUES.

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IN the course of experiments on a transplantable mouse sarcoma it was observed that fresh saline extracts of tumour tissue agglutinated mouse and rabbit red cells. When extract and red cell suspension were mixed on a slide agglutination became visible to the naked eye within 30 seconds and appeared to be complete within 2 minutes.

A number of different mouse and rat tumours, and a wide range of normal tissues, have been examined for haemagglutinating activity. The agglutinability of various species of red cells has been tested. Some physical and chemical properties of the haemagglutinating agents have been investigated.

Materials and methods.

Tumours.—

Mouse : 1. C57 sarcoma. 2. C57/LH1 sarcoma. 3. S37 sarcoma. 4. Crocker 180 sarcoma. 5. C57 X mammary adeno-carcinoma. 6. C57 A mammary adeno-carcinoma. 7. D13 thymoma.

Rat : 8. Walker 256 carcinoma. 9. Hepatoma.

Origin : No. 1, 2, and 6 were induced by methylcholanthrene. No. 3 arose as a sarcomatous transformation of stroma in a spontaneous carcinoma. No. 5 was induced by foster-nursing. No. 7 was a spontaneous tumour. No. 9 was induced by feeding butter-yellow. (Primary tumour only examined.)

Maintenance : No. 1, 2, 5, 6, and 7 were maintained by serial transplantation in C57 black mice, No. 3 and 4 in stock mice, and No. 8 in stock rats.

Preparation of extracts.—Tissue was ground in a " Tenbroeck " type grinder with Ringer, or buffered saline, to give 10 or 20 per cent suspensions. These were centrifuged at moderate speed ; the supernatant fluids contained the haemagglutinating agents.

Preparation of red cell suspensions.—Blood of various species was taken from a vein, or from the heart, into citrate solution, centrifuged, twice washed, and finally suspended in normal saline containing 0·024 per cent $CaCl_2$ (Burnet and Anderson, 1946). Commercial preparations of horse and sheep blood were used as sources of red cells of these species.

Haemagglutination tests.—Slide agglutination was used whenever qualitative information only was wanted, because of its simplicity and speed. When quantitative results were required a tube test based on that used by Burnet and his co-workers (Burnet, Beveridge, McEwin and Boake, 1945) was adopted.

Serial two-fold dilutions of tissue extract were made in calcium-saline in round-bottomed tubes of approximately 0·8 cm. internal diameter. Each tube received 0·1 c.c. of diluted extract and 0·05 c.c. of a 2 per cent suspension of washed red cells in calcium-saline ; the tubes were then shaken and allowed to stand at room temperature for 20–30 minutes. A preliminary reading of the end-point could then be made, if a quick answer was needed, which did not differ by more than one place from the final reading. Calcium-saline, 0·3 c.c., was then added, the tubes were shaken and allowed to stand a further $1\frac{1}{2}$ hours at room temperature, when the final reading was made.

The type of agglutination, and also of the deposition of red cells in saline controls, varied with species. Mouse cells seldom, and rabbit cells hardly ever, gave the compact button of deposit which is typical of unagglutinated fowl or human cells, and which workers with influenza virus haemagglutination take as negative. Rabbit cells, in the absence of an agglutinating agent, settle in a round-bottomed tube as an ill-defined deposit surrounded by a slightly granular film, which would be regarded as evidence of agglutination of fowl cells. It resembles closely the " shield pattern " which has been described (Burnet and Stone, 1946) as typical of agglutination of fowl cells by lipoids. Mouse cells, when unagglutinated, usually settle partly as a film, partly as a button or ring. In these tests, the last tube which showed granularity of the deposit definitely coarser than that of a saline control was taken as the end point. Higher con-

centrations of haemagglutinating agent caused the cells to coalesce into a reticular coagulum, which, on shaking, broke up into long shreds and large flakes floating in a clear fluid.

Very little haemolysis was seen, unless the test was allowed to stand for long periods at room temperature (24–48 hours) or was carried out at 37° C.

Preservation of haemagglutinating agent.

The haemagglutinating power of tumour extracts declined rapidly on standing. In the case of the C57 sarcoma, activity fell to about one-half its original value in an hour and had usually disappeared after 24 hours, at room temperature. Activity of other tumour extracts declined more rapidly.

An attempt was made to determine the cause of this decline of activity, and to find means of preventing it. The effect of adding various enzyme poisons to fresh tumour extracts was tried. Neither sodium azide (0·08 per cent), nor sodium iodoacetate (0·0001 to 0·003 M), nor sodium cyanide (0·002 M, neutralized), prevented or delayed the decline of haemagglutinating power of an extract of C57 sarcoma. Antoxidants were then tried. Ascorbic acid (0·01 M, neutralized), hydroquinone (0·01 M), and bright iron, were ineffective. Decline of activity was as rapid in an oxygen-free atmosphere (McIntosh and Fildes jar) as in air. SH compounds, however, had a definite retarding effect on the decline. Cysteine and glutathione (0·01 M, neutralized) both appeared to retard the decline of haemagglutinating activity appreciably, but their effect was difficult to estimate because they had slight haemagglutinating action themselves. Sodium thioglycollate (0·01 M) and 2,3 dimercapto propanol (British Anti-Lewisite, or B.A.L., 0·01 M) were much more powerful as preservatives and had no haemagglutinating action of their own. Both preserved the activity of tumour extracts at a constant level for long periods. Thioglycollate was found to be fairly rapidly haemolytic, even in high dilution (0·001 M or higher). B.A.L. proved a very satisfactory preservative. It was effective in low concentration (0·005 M), and though it increased slightly the granularity of deposits of red cells in control tubes, it had no haemagglutinating action which could be confused with that of tumour extracts, even in concentrations much higher than this. It was slowly lytic for mouse red cells (though this did not interfere with tests read after the usual period of two hours) but not for those of other species. A final concentration of 0·01 M (0·12 per cent) was generally used ; this was conveniently obtained by adding to a tumour extract 1/50th of its volume of a saturated aqueous solution of B.A.L. (approximately 6 per cent).

Besides preventing loss of activity of fresh extracts, B.A.L. brought about regeneration of the activity of inactive extracts to their original titre. This regeneration was progressive : some activity was apparent in a previously inactive extract a few seconds after the addition of B.A.L., but the full titre was not reached for about an hour, at room temperature.

Table I shows the preservation by B.A.L. of the haemagglutinating power of a C57 sarcoma extract for rabbit red cells.

Further observations on the action of B.A.L. are recorded in later sections.

Species range of haemagglutination.

No significant differences in agglutinability were found between red cells of normal and tumour-bearing mice, or between mice of different breeds. Red

cells of other species were agglutinated by tumour extracts in varying degree. The addition of sodium thioglycollate or B.A.L. to a tumour extract did not alter the species range of its agglutinating action.

TABLE I.—*Preservative and Regenerative Action of B.A.L. on the Haemagglutinating Power of a Sarcoma Extract.*

C57 sarcoma extract.

Containing B.A.L. 0·01 M, after standing 45 mins. at room temperature	64
,, ,, ,, ,, 4 hours ,, ,,	128
,, ,, ,, ,, 25 ,, ,, ,,	64
Not containing B.A.L., after standing 45 mins. at room temperature	32
,, ,, ,, 4 hours ,, ,,	8
,, ,, ,, 25 ,, ,, ,,	1
B.A.L. 0·01 M added after standing 25 hours at room temperature	64

Figures represent reciprocals of haemagglutinating titres for rabbit red cells.

Table II shows the results of agglutination tests between a C57 sarcoma extract and mouse, rat, guinea-pig, rabbit, horse, sheep, human, and fowl cells.

TABLE II.—*Agglutination of Various Species of Red Cells by a C57 Sarcoma Extract Containing B.A.L. 0·01 M.*

	Red cell species.								
	Mouse.	Rat.	Guinea-pig.	Rabbit.	Horse.	Sheep.	Human Group O.	Fowl, lipoid-agglutinable.	Fowl, lipoid non-agglutinable.
Reciprocal of agglutinating titre	8	Unstable	2	128	2	4	2	2	2

Lipoid suspensions, such as Kahn antigen, have been shown to agglutinate the red cells of some fowls, but not of all (Burnet and Stone, 1946). Fowl cells of each type, distinguished by a previous test against Kahn antigen, were included in this test.

It will be seen that rabbit cells were agglutinated by the tumour extract to far higher titre than those of any other species tried. Lipoid agglutinable and lipoid non-agglutinable fowl cells were agglutinated to the same low titre. Tumour extracts varied somewhat with regard to the ratio between titres for different red cells, but these variations were not constant. The titre for rabbit cells was always between 4 and 16 times the titre for mouse cells. Extracts treated in various ways, to be described later, were tested in most cases against rabbit and mouse cells. No significant alterations of the ratio of rabbit cell to mouse cell titres were observed.

Occurrence of haemagglutinating agents in various tumours.

Besides C57 sarcoma, other tumours, listed on p. 254, were examined for haemagglutinating agents.

The haemagglutinating titres of extracts of these tumours varied greatly (Table III). The sarcomata gave higher titres than the carcinomata.

TABLE III.—*Haemagglutinating Titres of 20 per cent Extracts of Various Mouse and Rat Tumours.*

	Red cells.	
	Rabbit.	Mouse.
C57 mouse sarcoma	32 to 256	8 to 64
C57/LH1 mouse sarcoma	16 ,, 64	—
S37 mouse sarcoma	4 ,, 64	2 to 16
Crocker 180 mouse sarcoma	++++	+++
D13 mouse thymoma	1 to 8	0 to 2
C57X mouse mammary carcinoma	2	0
C57A ,, ,, ,,	2	—
Walker 256 rat carcinoma	++++	++
Butter yellow rat hepatoma	8	1

Figures represent reciprocals of titres in the tube test.
+ to ++++ represent degrees of agglutination in the slide test.
All extracts contained B.A.L. 0·01 M.

C57 and S37 sarcoma extracts were compared in respect of red cell species range. They differed only in a manner which could be accounted for as a proportionately lower titre of S37 extracts for all species, e.g. red cells which were agglutinated to low titre by C57 were inagglutinable by S37 extracts.

Extracts of all the tumours tested lost their activity on standing, though at varying rates, e.g. extracts of C57 sarcoma took about 24 hours to become inactive, but extracts of C57/LH1, sarcoma, though initially often of similar titre, became inactive in one hour, or less. The loss could be prevented, or reversed, by the addition of B.A.L.

Occurrence and distribution of haemagglutinating agent in normal tissue.

Extracts of mouse liver, spleen, kidney, heart, lung, brain, voluntary muscle, lymph gland and thyroid, derived from both normal and tumour-bearing mice, were found not to agglutinate mouse red cells; and for a time it was thought that the haemagglutinating agent was confined to tumours. Examination of a wider range of normal tissues, using rabbit as well as mouse red cells, showed that this was not so.

Extracts of several tissues, notably adult mouse testicle, ovary, uterus and voluntary muscle, as well as whole mouse embryo and placenta, agglutinated rabbit red cells and sometimes mouse red cells also. The haemagglutinating titres of these extracts were, in general, low compared with those of extracts of mouse sarcomata, but comparable with those of extracts of some of the other tumours examined. Table IV gives the range of haemagglutinating titres for rabbit, mouse and human red cells, of a number of normal tissue extracts. The haemagglutinating power of these extracts declined on standing, and was preserved, or regenerated, by the addition of B.A.L. in the same way as that of tumour extracts (Table V). Their species range of haemagglutination showed no difference from that of tumour extracts which could not be accounted for as a proportionately lower titre for all species.

TABLE IV.—*Haemagglutinating Titres of 20 per cent Extracts of Normal Mouse Tissues.*

	Red cells.		
Positive :	Rabbit.	Mouse.	Human.
Uterus, oestrous	32	4	0
,, dioestrous	8	2	0
,, anoestrous	8	2	0
Whole embryo, 19–20th day	8	1	0
Placenta ,, ,,	8	1	0
Testicle	4	0	0
Ovary	2	0	0
Voluntary muscle	8	0	0
Skin	4	0	0
Large intestine	8	0	0

Negative :

Brain, heart, lung, liver, spleen, kidney, thyroid, seminal vesicles, lymph-gland. Stomach and small intestine extracts were too strongly haemolytic for testing.

All extracts contained B.A.L. 0·01 M.
Figures represent reciprocals of titres.

TABLE V.—*Preservative and Regenerative Action of B.A.L. on the Haemagglutinating Power of Mouse Embryo Extract.*

Extract of 20-day embryos.

Containing B.A.L. 0·01 M, after standing 45 mins. at room temperature	8
,, ,, ,, ,, 22 hours ,, ,,	8
Not containing B.A.L., after standing 45 mins. at room temperature	4
,, ,, ,, 2 hours ,, ,,	0
,, ,, ,, 22 ,, ,, ,,	0
B.A.L. 0·01 M added after standing 22 hours at room temperature	8

Figures represent reciprocals of haemagglutinating titres for rabbit red cells.

Some other properties of the haemagglutinating agents of tumours and normal tissues.

Action of normal serum on haemagglutination.—Haemagglutination by lipoids is inhibited by normal serum in high dilution, while haemagglutination by viruses is unaffected by normal sera, but inhibited by specific antiviral sera (Burnet and Stone, 1946).

The effect of normal horse serum on haemagglutination by tumour and normal tissue extracts was tested. Extracts were mixed with equal volumes of a 1 in 100 dilution of horse serum, and the mixtures tested for haemagglutination in the usual way. No inhibition was observed.

Action of sera of mice in which tumours had regressed on haemagglutination.—S37 sarcoma grafts grow for a time in C57 black mice and then regress. Similarly, C57 sarcoma grafts grow for a time and then regress in stock albino mice. Serum was collected from four C57 black mice 26 days after grafting with S37 sarcoma

and from four stock albino mice 23 days after grafting with C57 sarcoma, at which times only small residual nodules remained. An experiment similar to the last showed that addition of either of these sera had no effect on haemagglutination by either C57 or S37 sarcoma extracts.

Effect of temperature at which the test is performed on haemagglutination.—It has been shown that haemagglutination by lipoids and by vaccinia virus is more active at 37° C. than at room temperature (Stone, 1946; Burnet and Stone, 1946), while haemagglutination by influenza virus is more active in the cold than at room temperature or 37° C. (Salk, 1944).

Haemagglutination by extracts of C57 and S37 sarcomata, and of normal mouse embryo and adult uterus, was tested at approximately 4° C., at room temperature, and at 37° C. The process of haemagglutination was actually accelerated by increase of temperature in all cases, but this did not result in a higher titre as judged by reading after the usual two-hour period. Table VI

TABLE VI.—*Effect on Haemagglutination of the Temperature at which the Test is Performed.*

	Temperature of test.		
	+ 4° C.	Room temperature.	37° C.
C57 sarcoma extract	64	64	32
S37 „ „	32	32	16
Mouse embryo extract	2	2	4
Mouse uterine „	16	32	16

Figures represent reciprocals of haemagglutinating titres for rabbit cells. All extracts contained B.A.L. 0·01 M.

shows that the titres of tumour extracts fell slightly with increase of temperature, a change in the same direction as that reported for influenza virus, though much smaller. The slight variations shown in titre of normal tissue extracts at different temperatures are probably not significant.

Neither tumour nor normal tissue extracts resemble lipoids in this respect.

Action of complement on haemagglutination.—A standard quantity of an extract of C57 sarcoma (16 haemagglutinating doses) was tested against rabbit and mouse red blood cells, with and without the addition of guinea-pig serum (approximately 10 M.H.D. measured in a sheep cell-antisheep cell serum system). The mixtures were held at 37° C. for ¾ hour, then at 4° C. overnight. The presence of complement had no detectable effect: neither rabbit nor mouse cells were lysed and their agglutination by the tumour extract was unaffected.

Absorption of haemagglutinating agent by red cells.—C57 sarcoma extracts were absorbed with rabbit, mouse, and fowl red cells, under various conditions. The following is a representative experiment.

Samples of 20 per cent extracts of C57 sarcoma, (i) fresh, i.e. 1 hour after making, (ii) inactive, i.e. after standing overnight, and (iii) preserved by B.A.L. (0·01 M) were absorbed with 1/10th of their volumes of packed rabbit, mouse, and fowl red cells.

Mixtures (i) were held at room temperature for 30 minutes only, in order to minimize possible re-dispersion of the red cells owing to decline of activity of the

extract, and then centrifuged. Mixtures (ii) and (iii) were held at 4° C. overnight, and then centrifuged. The supernatant fluids were removed and the cells re-suspended in the original volumes of saline. The re-suspended cells were incubated at 37° C. for three hours, and centrifuged; the supernatant fluids were removed and the cells finally resuspended in the original volumes of saline, and examined for persistent agglutination and for agglutinability. B.A.L. was added to 0·01 M to all the supernatant fluids and they were tested for haemagglutinating power.

The results may be summarized as follows:

Haemagglutinating agent was absorbed by red cells when, and only when, they were agglutinated by it. Rabbit and mouse cells, in the doses used, absorbed about ¾ of the haemagglutinating agent present in fresh, or in B.A.L.-preserved extracts (i.e. extracts which agglutinated them strongly). Neither absorbed any agent from an extract which had become inactive, though presence of the agent in the extract was proved by subsequent addition of B.A.L. Fowl cells, which were only very slightly agglutinated, did not absorb appreciably.

In all except one case red cells agglutinated during absorption were unsuitable for subsequent tests for agglutinability because they remained agglutinated. The exception was mouse cells used to absorb fresh extract. These, though agglutinated in the presence of the extract (30 minutes at room temperature) redispersed during the subsequent incubation with saline. They were then found to be fully agglutinable by tumour extract containing B.A.L., but not by B.A.L. alone. Mouse and rabbit cells which did not agglutinate during absorption, i.e. those used to absorb inactive extract, were also found to be subsequently fully agglutinable by a B.A.L.-containing tumour extract, but not by B.A.L. alone.

Since red cells after absorption, when they were suitable for subsequent agglutination tests, proved agglutinable by the same tumour extract which they had been used to absorb, it was not thought worth while to attempt cross-agglutination between different extracts (e.g. those of different tumours, or of tumour and normal tissues) and red cells used to absorb them.

Partial release of haemagglutinating agent occurred during incubation of red cells in saline after absorption. Recovery was never complete but was most nearly so in the case, noted above, of mouse cells used to absorb fresh tumour extract.

A good deal of haemolysis of agglutinated red cells occurred; this was noticeable during absorption and during the subsequent incubation in saline. Haemolysis is not appreciable in haemagglutination tests, and its appearance in absorption mixtures was unexpected. The difference is probably quantitative. Gross (1947) has reported the presence of haemolytic agents in several tumours. Haemolysis by such an agent, if of low titre, would be active only in the first one or two tubes of a haemagglutination test and, since these contain only 1/450th of their volume of packed red cells, haemolysis might be masked by the small amount of haemoglobin present in all crude tissue extracts. But in absorption mixtures consisting of undiluted extract and 1/10 volume of packed red cells, even slight haemolysis would be obvious.

Filtration and dialysis.—The haemagglutinating agent of tumour extracts containing B.A.L. passed through Gradocol membranes of A.P.D. 0·6 to 0·7μ, and through Berkefeld V candles, with only slight loss of activity. In the absence

of B.A.L., activity seemed to be lost even more rapidly during filtration than on standing, but reappeared as usual after addition of B.A.L. to the filtrate. The agent was not dialysable through cellophane.

At an early stage in this work, when it was thought, on the basis of preliminary tests, that the haemagglutinating agent was confined to tumour tissue (p. 257), a number of mice were injected with cell-free filtrates of tumour extracts containing B.A.L., on the assumption that the haemagglutinating agent might be associated with a labile filtrable tumour-producing agent.

Cell-free haemagglutinating filtrates of saline or broth extracts of C57, S37, and Crocker 180 mouse sarcomata, containing B.A.L. 0.005 to 0.01 M, were injected subcutaneously and intraperitoneally into groups of 4 to 8 mice of appropriate breeds. In several cases mixtures of filtrate and mouse red cells were injected. The mice were observed for 4 to 10 weeks. No tumours developed. To exclude the possibility that failure to produce tumours was due to inhibition of growth by B.A.L., a piece of S37 sarcoma was minced in broth containing 0·005 M B.A.L., and kept at 4° C. for 48 hours before grafting into mice. Grafts of the same tumour minced and kept in plain broth were made into control mice. Both groups developed tumours; those which grew from the B.A.L.-treated grafts lagged behind at first but, by the eighteenth day, there was no difference in size of tumours between the groups.

Centrifugation.—The haemagglutinating agent of a C57 sarcoma extract was not deposited by centrifugation in an angle head at 9000 r.p.m. for 1½ hours. High speed centrifugation has not been tried.

Precipitation by ammonium sulphate.—The haemagglutinating activity of a C57 sarcoma extract was associated with a fraction precipitated between $\frac{1}{6}$ and $\frac{3}{4}$ saturation with ammonium sulphate, and most of it with a fraction precipitated between $\frac{1}{4}$ and $\frac{5}{8}$ saturation.

Action of heat.—C57 sarcoma extract was heated at 55° C. for 30 minutes and at 100° C. for 10 minutes. At pH between 7 and 8 the former treatment was found to reduce the haemagglutinating titre to about half, the latter to about one-eighth. At pH 6, and at pH 9, boiling for 10 minutes destroyed all activity. There was no difference between titres of extracts to which B.A.L. had been added before, and those to which it had been added after, heat treatment.

A heavy precipitate formed in the heated extract, which was spun out before testing for haemagglutinating power, and it was thought possible that the haemagglutinating agent might be adsorbed on the precipitate. However, no agent could be recovered by extracting the precipitate with M/15 Na_2HPO_4.

Action of enzymes.—Trypsin and Papain. The effect of digestion of tumour extracts by trypsin or papain depends on whether they contain B.A.L. In the absence of B.A.L., extracts of C57, or of C57/LH1, sarcoma incubated at 37° C. with trypsin or papain (activated with KCN) for an hour, were found to be irreversibly inactivated, i.e. subsequent addition of B.A.L., even up to 0·1 M, caused no return of haemagglutinating power. Addition of B.A.L. 0·01 M to the extracts before digestion prevented any loss of haemagglutinating power for at least 2½ hours, but after 20 hours' digestion a considerable drop in titre was observed, which was not reversible by further addition of B.A.L.

These results are illustrated in Table VII A, which records the effect of digestion of C57 sarcoma extract by trypsin. Digestion by papain had essentially similar effects.

Table VII.

A. *Action of Trypsin on Haemagglutinating Agent of Sarcoma Extract, and its Inhibition by B.A.L.*

	Time of incubation. 1 hour.	2½ hours.	20 hours.
1. 20 per cent extract of C57 sarcoma, control	16	8	4
2. The same + B.A.L. 0·005 M	32	32	32
3. „ + trypsin	0	0	0
4. „ + „ + B.A.L. 0·005 M	32	16	4
5. „ + „ + „ 0·01 M	32	32	4

Conditions: Incubation 37° C. Trypsin 0·5 per cent of commercial powder (Hopkins & Williams). All mixtures buffered at pH 8 with M/15 phosphate, 2 drops of chloroform added as bacteriostatic; neutralized, and B.A.L. added to 0·01 M, before test.

Figures represent reciprocals of haemagglutinating titres for rabbit red cells.

B. *Proteolytic Action of Trypsin on Sarcoma Extract in Relation to its Action on the Haemagglutinating Agent, and Inhibition of Both Actions by B.A.L.*

	Time of incubation 2½ hours. Haemagglutinating titres.	Mg. amino N per 5 c.c., excess over control.
1. 20 per cent extract of C57 sarcoma, control	64	—
2. The same + trypsin	0	3·43
3. „ + „ + B.A.L. 0·005 M	64	1·82
4. „ + „ + „ 0·01 M	64	1·61

Relevant conditions as in A.

C. *Inhibition by B.A.L. of Tryptic Digestion of Casein.*

4 per cent solution of casein,	Time of incubation. 1 hour.	2½ hours.	Mg. amino N per 5 c.c., excess over control.
1. + trypsin	0·43	1·54	
2. + „ + B.A.L. 0·005 M	0·57	0·84	
3. + „ + „ 0·01 M	0·43	0·56	

Casein was "light, soluble" of British Drug Houses, dissolved in M/15 Na_2HPO_4 by heating 1 hour on a water-bath.

Relevant conditions as in A.

It will be noted that there was a considerable irreversible drop in titre of a control sample of sarcoma extract incubated without B.A.L. (Table VII A, No. 1), while that of a similar sample incubated with B.A.L. (0·005 M) remained constant (Table VII A, No. 2). A similar drop has been observed during storage at 4° C. of extracts not containing B.A.L., i.e. the titre attainable by addition of B.A.L. to such extracts gradually falls. It seems probable that this effect is due to the action of tissue proteases.

The effect of tryptic digestion on extracts of normal mouse tissues was also tried. It is difficult to obtain such extracts with haemagglutinating titres high enough to make this test satisfactory. In an experiment in which a mouse embryo extract with a haemagglutinating titre for rabbit cells of 1:4 was digested with trypsin, with and without B.A.L., activity appeared to be destroyed in

both cases. However, not much reliance can be placed on this result, because haemolysis by trypsin was rapid in the low dilutions, and may have masked haemagglutination.

The results of digestion of tumour extracts suggest that B.A.L. may act as an inhibitor of proteolysis by trypsin and papain. However, Webb and van Heyningen (1947) state that B.A.L. (0·0042 M) does not inhibit digestion of casein by either trypsin or papain, as judged by formol-titration of amino-acid nitrogen, and in certain circumstances activates papain.

Because of this apparent anomaly, the action of B.A.L. on proteolysis was examined, using tumour extracts, and casein, as substrates.

A 20 per cent extract of C57 sarcoma in M/15 phosphate buffer pH8 was incubated (*a*) alone, (*b*) with trypsin, (*c*) with trypsin, and B.A.L. 0·005 M, and (*d*) with trypsin and B.A.L. 0·01 M. The former concentration of B.A.L. was chosen because it has a definite, though sub-maximal, preservative effect on the haemagglutinating agent, and is approximately equal to that used by Webb and van Heyningen, the latter because it has the maximal preservative effect. After 2½ hours the mixtures were tested for haemagglutinating power, and their amino-nitrogen content determined by formol-titration.

In a similar experiment, a 4 per cent solution of casein ("light, soluble," British Drug Houses) in M/15 phosphate buffer pH8 was substituted for the tumour extract. Amino-nitrogen was determined after 1 and 2½ hours' incubation.

The results of these experiments are given in Table VII B and C. They show that, with casein as substrate, after 1 hour's incubation there was no detectable inhibition of proteolysis by B.A.L.; but with both casein and tumour extract as substrates, after 2½ hours' incubation there was definite inhibition: about 50 per cent by B.A.L. 0·005 M, and 60 per cent by 0·01 M. Both concentrations of B.A.L. protected the haemagglutinating agent from the action of trypsin for 2½ hours.

The action of crystalline trypsin, kindly supplied by Dr. S. D. Elliott, on the haemagglutinating power of a tumour extract, with and without B.A.L., was essentially similar to that of commercial trypsin.

The haemagglutinating agent does not become dialysable through cellophane after digestion of a B.A.L.-containing tumour extract with trypsin.

Lecithinase: It has been shown by Stone (1946*a*) that the haemagglutinating action of lipoids, and of vaccinia and ectromelia viruses, which probably owe their haemagglutinating action to lipoid components, is destroyed by *Cl. welchii* toxin, which contains an active lecithinase.

A sample of partially purified *Cl. welchii* toxin was kindly supplied by Miss M. G. Macfarlane. A preliminary test against egg-yolk (the lecitho-vitellin test of Macfarlane, Oakley and Anderson, 1941) showed that this preparation was highly active. The addition of B.A.L. (0·01 M) to the substrate greatly retarded the action of the enzyme, as judged by this test. Accordingly, its action on tissue extracts not containing B.A.L. was tried. To C57 sarcoma extract, buffered at pH 7·6 by the addition of borate buffer, and containing $CaCl_2$ 0·01 M (Stone, 1946*a*), *Cl. welchii* toxin was added to give a final concentration of 1·2 lecithinase units per c.c. (The unit of lecithinase, defined by Macfarlane and Knight (1941) is that amount which, under defined conditions, produces 0·1 mg. acid-soluble P from lecithin in 15 minutes at 37° C.) The mixture was incubated

for 2½ hours at 37° C. B.A.L. to 0·01 M was then added, and haemagglutinating power tested. In a similar experiment mouse embryo extract was treated with the toxin.

The results showed that incubation with lecithinase had no detectable effect on the haemagglutinating agents of C57 sarcoma or mouse embryo extracts. Haemolysis by lecithinase present in the mixtures was not rapid enough, in the presence of B.A.L., to interfere with haemagglutination.

Action of heavy metals and oxidizing agents, and its reversal by B.A.L.—To samples of fresh, actively haemagglutinating, sarcoma extract, not containing B.A.L., $CuSO_4$ was added, to give final concentrations of M/300 and M/3000. In both mixtures haemagglutinating activity disappeared within a few seconds; there was a partial return of activity in the later, but none in the former, after addition of B.A.L. to 0·01 M. Manganese, cobalt, and ferric salts, had similar but less powerful effects in the same concentrations, which were also partially reversible by B.A.L. In order to determine whether the natural inactivation of the agent is due to the action of heavy metals, samples of a fresh sarcoma extract, not containing B.A.L., were saturated with benzoin-oxime ("Cupron") and 8 hydroxy-quinoline respectively. The former substance reacts specifically with copper, the latter with any heavy metal, forming inactive compounds. The haemagglutinating activity of these mixtures declined on standing at the same rate as that of a control. It thus appeared that the presence of heavy metals was not essential for natural inactivation.

The addition of various oxidizing agents to a fresh sarcoma extract destroyed or reduced its haemagglutinating activity. Hydrogen peroxide (10 per cent of "hydrogen peroxide 100 vols." Boots), sodium hypochlorite (10 per cent of a 1 per cent solution—"Milton"), and iodine (M/300) all destroyed the activity completely, while lower concentrations produced a drop in titre. Activity was restored, to a degree depending on relative concentrations, by the addition of B.A.L.

DISCUSSION.

A number of substances of widely various type and origin have the power to agglutinate red cells. The list includes serum antibodies (naturally occurring, acquired, or experimentally induced), many viruses (Hirst, 1941; Nagler, 1942; Burnet, 1945; Lush, 1943; Burnet and Stone, 1946; Mills and Dochez, 1944), purified lipoids (Stone, 1946*b*), bacterial extracts (Keogh, North, and Warburton, 1947), the globulin fraction of egg-white (Commission on Acute Respiratory Diseases, 1946), some plant proteins such as ricin and abrin, basic proteins such as protamines and histones, inorganic colloidal acids and bases such as colloidal silicic acid, and many metallic salts (Landsteiner, 1945). No report has appeared, as far as the author is aware, of haemagglutination by extracts of tissues, either normal or neoplastic. Gross (1947, 1948) has described haemolysis of mouse, but not rabbit, guinea-pig, or human red cells, by extracts of mouse mammary carcinoma and lymphatic leukaemia tissue, both spontaneous and grafted, but he does not report the occurrence of haemagglutination. It is perhaps not surprising that haemagglutination by tissue extracts has not been hitherto observed, in view of the lability of the agents. It was a fortunate accident that C57 sarcoma was the first tumour to be examined, extracts of which

remain active for several hours. But for this, the phenomenon would probably not have been noticed, for the majority of tumour and normal tissue extracts examined remain haemagglutinating for a much shorter time, and then only for rabbit cells. Their study would not have been possible without experience gained with C57 sarcoma.

The tissue haemagglutinating agents differ from both viruses and lipoids in various ways. They are labile, while the latter are both fairly stable substances; their red-cell species specificity is different from that of both lipoids and viruses; they are unaffected by *Cl. welchii* toxin, which destroys the haemagglutinating power of lipoids and of vaccinia and ectromelia viruses; their action is unaffected by normal serum, which inhibits that of lipoids but not that of viruses. In the manner in which temperature affects haemagglutinating titre they differ from lipoids. A sarcoma extract is absorbed by red cells which it agglutinates, but the cells, when they redisperse, as they do under certain conditions, are fully agglutinable by the same agent; red cells agglutinated by a virus may also redisperse, but are then inagglutinable by that virus, and often by some others also (Burnet, Beveridge, McEwin and Boake, 1945).

Egg-white is more like the tissue extracts in its haemagglutinating properties than are the other haemagglutinating substances referred to, particularly in respect of red cell species range, and effect of temperature on haemagglutinating titre, but it is not reported to be labile.

The general properties of the haemagglutinating agent of tumours suggest that it is a readily oxidizable substance which is active only in the reduced state. It may be a sulphydryl compound, but there is at present no positive evidence of this. Neither inactivation by heavy metals, nor by oxidizing agents, is conclusive on this point. The former may act by forming compounds with sulphydryl groups, or by catalyzing oxidation; the latter may act on sulphydryl or other oxidizable groups. More evidence is needed, also, before the nature of the preservative action of B.A.L. and other sulphydryl compounds can be determined. However, the fact that other reducing agents, e.g. ascorbic acid, are ineffective, suggests that preservation is a specific effect of sulphydryl. Natural inactivation does not appear to depend on the presence of heavy metals in an active state, and therefore it is unlikely that its prevention by B.A.L. is due to the latter's power of combining with them.

The protection of the haemagglutinating agent by B.A.L. from destruction by proteolytic enzymes is difficult to understand on the basis of available knowledge. B.A.L. has been stated not to inhibit the action of trypsin or papain (Webb and van Heyningen, 1947), but this work has been repeated here, with a different result. It has been shown that B.A.L. 0·005 M produces 50 per cent inhibition of proteolysis of casein, or tumour extract, incubated with trypsin for 2½ hours. During this period B.A.L. protects the haemagglutinating agent from the action of trypsin, whereas in the absence of B.A.L. it is completely destroyed.

A fuller understanding of these facts must await the result of further work.

Attempts to find qualitative differences between the haemagglutinating agents of normal tissues and those of tumours, or between those of different tumours, have been unsuccessful. The observed differences are either so small as to be of doubtful significance, or can be regarded as quantitative. Though the possibility that different types of agent exist has not been excluded, there is no positive evidence of this at present.

SUMMARY.

Extracts of mouse tumours agglutinate rabbit, mouse, and some other, red cells.

Some normal tissue extracts agglutinate rabbit cells; a few agglutinate mouse cells also. Extracts of mouse embryo, placenta, uterus, testicle, ovary, voluntary muscle, skin, and large intestine, have slight but definite activity, those of mouse brain, heart, lung, liver, spleen, kidney, thyroid, seminal vesicles, and lymph glands, have none.

Rabbit red cells are the most sensitive to agglutination of those tested. Mouse cells are agglutinated to titres four to sixteen times lower than rabbit; sheep, horse, guinea-pig, human, and fowl cells two to four times lower than mouse.

The haemagglutinating agents of tumours and of normal tissues lose their activity rapidly on standing. Addition of sulphydryl compounds, notably 2:3-dimercapto-propanol (B.A.L.) preserves their activity, or regenerates it after loss. Loss of activity is not prevented by addition of other reducing agents, or of enzyme poisons, or by storage in an oxygen-free atmosphere.

Certain physical and chemical properties of the haemagglutinating agent of tumours are described. Reasons are given for regarding it as an easily oxidizable substance, active only in the reduced state. It is destroyed by proteolytic enzymes, but not by lecithinase; B.A.L. protects it from the former.

B.A.L. was found to have definite inhibitory action on proteolysis by trypsin, and on digestion of egg-yolk by lecithinase.

The haemagglutinating agents of tumours and of normal tissues differ from haemagglutinating viruses and lipoids in important respects.

No qualitative differences have been found between the haemagglutinating agents of different tumours, or between those of tumours and normal tissues.

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OESTROGEN-INDUCED FIBROIDS OF THE THORACIC SEROSA.*

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UTERINE fibroids have been induced in the guinea-pig by the prolonged administration of oestrogen (Nelson, 1937*a*, 1937*b*; Moricard and Cauchoix, 1938). This finding acquired a more general interest from the point of view of the tumorigenic action of oestrogen in the body when it was discovered that fibroids can be elicited in this species not only on the uterus but on multiple spots of the abdominal serosa (Lipschutz and Iglesias, 1938). It was found that the incidence of these abdominal fibroids can reach up to about 80 per cent or more in female guinea-pigs to which oestrogen is administered continuously for three months (Lipschutz, 1942; Lipschutz and Vargas, 1939). Argentine workers found fibroids on the thoracic side also of the diaphragm in 2 out of 30 animals (Sammartino and Gandolfo, 1940). On the contrary, thoracic fibroids were extremely rare in our own work; it was the same in the extensive work of Von Wattenwyl (1944) with oestrogen-induced abdominal fibroids.

The question arises whether the difference in behaviour of the abdominal and thoracic serosa towards oestrogen is due to a genetically fixed differential reactivity of the endothelial or subendothelial cells of the two serosal covers, or to an internal "ambiental" factor as represented by the abdominal serosal fluid. Results with the experimental proof of the second of these possibilities are given in the present paper.

The above question seems all the more important on account of the following finding. In the male guinea-pig the inguinal channel remains open throughout

* Part of this material has been used by H. Elgueta for his Thesis of M.D. in 1944, for which the Award of the "Liga Nacional del Cáncer" has been received.

the whole life, and when oestrogen is administered, small fibrous nodules appear not only on the abdominal serosa but also on the surface of the testicle.

Incidence of oestrogen-induced thoracic fibroids.

Thoracic tumours were found in this Department in 3 out of 1310 females, and in 2 out of 147 males, in which abdominal fibroids were induced by natural or synthetic oestrogens. These findings are summarized in Table I.

TABLE I.—*3 Females and 2 Male Guinea-pigs with Oestrogen-induced Thoracic Fibroids, out of* 1310 *Females and* 147 *Males with Abdominal Fibroids. Two Additional Animals with Minor Serosal Reactions.*

Group.	Duration of treatment (days).	Oestrogen used.	Localization of thoracic tumours.	Histological description.
Castr. females:				
LXXII.40	96	Oestradiol subcutaneous tablet	Parieto-diaphragmatic	Fibroid with advanced sclerosis; patch of oedematous tissue; probably smooth muscle fibres; proliferation of vascular endothelium; invasion of diaphragm; hypertrophic endothelium on the surface of the tumour.
CXXXI.28	99	Oestradiol, subcutaneous tablet	Diaphragmatic	Thick fibrous strands invading the muscular part of the diaphragm; thickening of diaphragmatic serosa.
XXI.56	218	Oestradiol-17-capryl., injections	Diaphragmatic, parietal and pulmonary (Fig. 1)	Typical fibroid but advanced sclerosis; hyperplastic endothelium of vascular spaces.
Castr. males:				
LIX.73	279	Oestradiol dipropionate, subcutaneous tablet	Pulmonary	Not examined.
XCIII.58	813	Diethylstilboestrol, subcutaneous tablet	Pedunculated tumour, fixed to the trachea (Fig. 2)	Sclerosed tissue; probably proliferation of vascular endothelium.
XCIII.34	562	Diethylstilboestrol, subcutaneous tablet	Pulmonary	Sclerotic nodule; maximum diameter, about 0·2 mm.
XCIII.69	734	Ditto.	,,	Small cyst of pulmonary pleura; thick fibrous wall.

In 2 of the 3 females the tumours were present both on the diaphragm and the thoracic wall (Fig. 1). The parietal tumours are sometimes found at a considerable distance from, and in no connection with, the diaphragm. A tumour in the male was attached to the trachea (Fig. 2). The histological structure of the thoracic tumours was coincident with that of oestrogen-induced abdominal fibroids. The peripheral belt of fibroblasts originating most probably from subendothelial cells of the serosa and so characteristic of the oestrogen-induced abdominal fibroid (Lipschutz, 1942) was present in a diaphragmatic tumour belonging to the animal of Fig. 1. But collagenous fibres prevailed in

the three tumours examined (Table I). As with the abdominal fibroids we have described formerly, there were in thoracic tumours also some cells resembling smooth muscle fibres. There was a proliferation of the cells of the wall of vascular spaces; there was an hypertrophy of the endothelium at some places on the surface of the tumour. There was invasion of the muscular tissue of the diaphragm, or the thoracic wall, by the tumours. The pedunculated tumour belonging to the animal of Fig. 2 necropsied after 813 days of action of the oestrogen, consisted almost exclusively of hyalinized collagenous fibres, as do abdominal fibroids in experiments of long duration or after the withdrawal of oestrogens (Lipschutz and Schwarz, 1944). But there were in this tumour also accumulations of cells resembling fibroblasts which seemed to originate from the proliferation of the subendothelial cells of the wall of vascular spaces, or the endothelium proper. The tumour was covered by various layers of flattened cells.

There were besides the animals described, 4 females and 2 males in which small nodules just visible to the naked eye were found on the surface of the lung or on the parietal pleura. Only 2 animals of this group have been examined microscopically (Table I) and in one of these a tiny fibrous nodule was found on the surface of the lung; the structure of this nodule was identical with that of similar oestrogen-induced nodules of the abdominal serosa. Even when these additional animals are taken into consideration the incidence of thoracic fibroids remains insignificant when compared with that of abdominal fibroids: a total of 11 animals with fibroids or some fibrous reaction in the thoracic cavity as against a total of 1457 animals with fibroids in the abdominal cavity, or less than 1 animal with a thoracic reaction per 100 animals with an abdominal reaction.

EXPERIMENTAL PROCEDURE.

Communication between the abdominal and thoracic cavity.

A circular piece of about 1·5 to 2 cm. in diameter was cut out in the left half of the diaphragm by abdominal approach. Mortality was considerable; only 39 out of 66 animals survived for more than a month. Administration of oestrogen began at 30 days after the operation, when a tablet containing 40 per cent of diethylstilboestrol and 60 per cent of cholesterol, or a tablet of oestradiol dipropionate, was implanted beneath the skin. At necropsy the opening was found closed again in about a third of the animals. These animals were omitted when comparing the results. In most of the remaining animals part of the liver or of the intestine, or of both, was found in the thoracic cavity. But the passage between the two cavities was never obstructed by the hernia, though there were partial adhesions of the prolapsing structure to the thickened sclerosed border of the opening. There were 18 animals which survived for 96 to 186 days.

As was to be expected, abdominal fibroids were present in all animals. In 7 out of the 18 animals there was also a thoracic fibrous reaction (Fig. 3). In 2 out of the 7 animals there were tumours on the diaphragm; fibrous nodules of the serosa at a distance from the diaphragm were present in all animals. It is remarkable that in 5 animals the thoracic nodules were present not only on the left side, where the opening was made, but also on the right side.

The histological structure of these thoracic tumours was identical to that of oestrogen-induced abdominal fibroids.

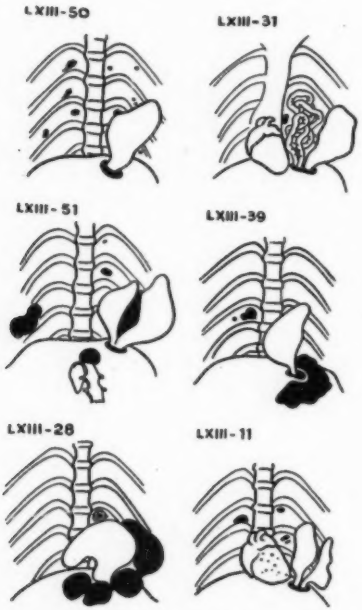


FIG. 3.—Diagram showing oestrogen-induced fibrous tumoural reaction in 6 animals with a communication between the thoracic and abdominal cavities. Note thickened border of opening in the diaphragm, prolapsed liver and intestine. Fibrous reaction also on the right though the opening is on the left. Small nodules on the pericardium in LXIII.11. Experiments with stilboestrol lasted: No. 50, 113 days; No. 31, 128 days; No. 51, 152 days; No. 39, 155 days; No. 28, 185 days. Experiment with oestradiol dipropionate: No. 11, 96 days.

Experiments with local stimulation of the thoracic serosa.

The above results leave no doubt that the incidence of oestrogen-induced thoracic fibroids was enormously increased by the procedure adopted. But the interpretation of the results is not an easy one. First of all there were the pro-

LEGENDS TO FIGURES.

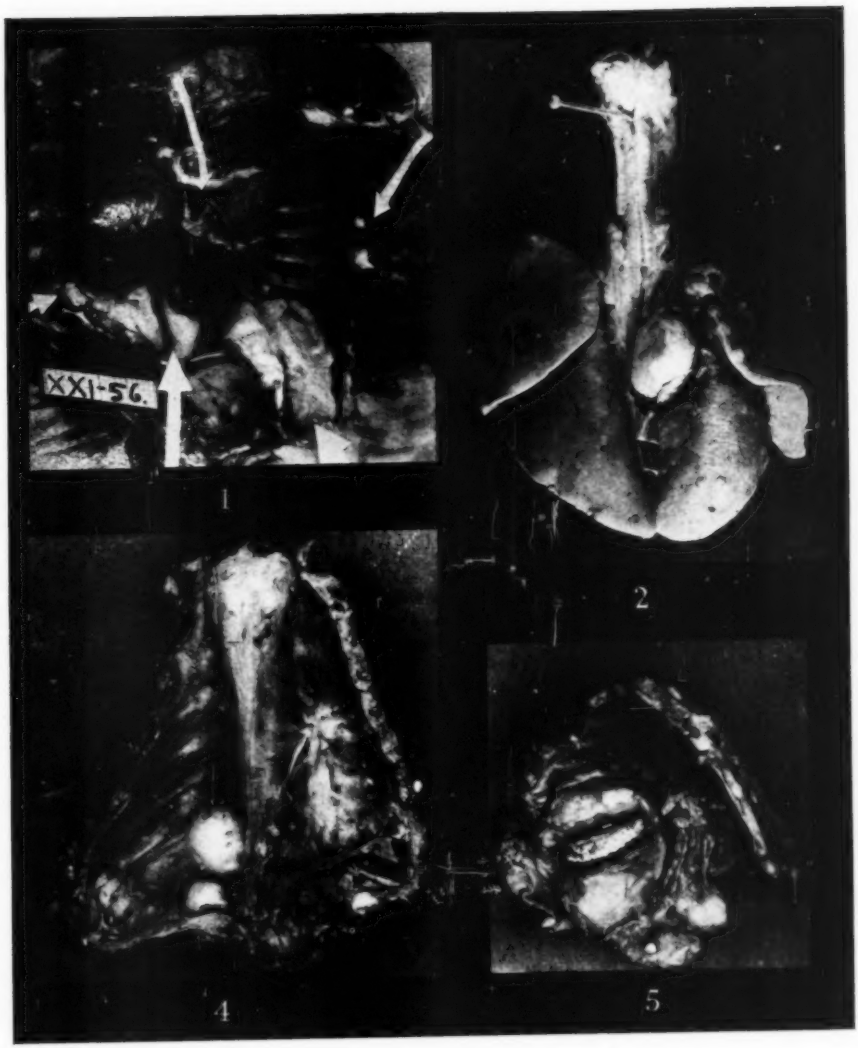
FIG. 1.—Thoracic cavity of female guinea-pig (XXI.56). 91 injections of 10 μg. of oestradiol (17-caprylate) in the course of 218 days. Various large fibroids on the thoracic surface of the diaphragm. Smaller fibroids on the parietal pleura of the left cavity. Also fibrous thickening of the parietal pleura. Minute nodules on the lung.

FIG. 2.—Male guinea-pig. Dorsal view (XCIII. 58). Absorption of stilboestrol from subcutaneously implanted tablet during 813 days. The pedunculated fibroid is attached to the trachea.

FIG. 4 and 5.—Thoracic cavity of female guinea-pig (LXIII.60). Absorption of 8 μg. of stilboestrol daily from subcutaneous tablet during 167 days. Opening between thoracic and abdominal cavities on the right side.

FIG. 4.—Two fibroids of the right parietal serosa and on the diaphragm. Large tumour and fibrous strands of the left thoracic cavity.

FIG. 5.—Large tumour of the left thoracic cavity opened; metallic tablet therein.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lapsed liver and intestines which might have acted as mechanic stimuli. It is known from the work of this Department that a metallic tablet or a glass bead, when introduced into the abdominal cavity, is fixed at the epiploon or other places of the abdominal cavity; when oestrogen is administered for a sufficient length of time the thin capsule surrounding the tablet is greatly thickened and the foreign body is found in the centre of a fibroid (Manhood, 1942; Monreal, 1944). It was thought that implantation of a metallic tablet into the thoracic cavity of animals to which oestrogen is administered might help to clear the question whether the supposed local stimulation could have been in play in the experiments described above.

First group of experiments.—A metallic tablet was introduced in the right thoracic cavity of 10 females. Simultaneously another metallic tablet was put into the abdominal cavity, and a tablet of stilboestrol was implanted beneath the skin. Necropsy was made 157 to 173 days later. The intrathoracic metallic tablet was found between the sternum and the diaphragm. It was covered with a rather thick capsule and there were fibrous strands in its surroundings, on the diaphragm, the costal and pulmonary serosa and the pericardium. But thoracic tumours as present in animals with a thoracic-abdominal communication were completely absent. In one animal only the capsule was thickened in such a manner as to become comparable to the tumours around the metallic tablet in the abdominal cavity of animals of this group.

Second group of experiments.—In another group of 14 animals a tablet of α-oestradiol or of oestradiol dipropionate was introduced into the thoracic cavity and necropsy was made 91 to 126 days later. The tablet occupied in some cases a retrosternal or mediastinal position; it was surrounded by a thin capsule and thin fibrous strands. In other cases the capsule adhered to the costal serosa, where the fibrous reaction around the oestradiol tablet was more conspicuous. But there was not a single animal with thoracic tumours in this group. On the contrary, in 12 out of these 14 animals there were abdominal fibroids, the degree of the fibrous reaction not being different from what is usual with a subcutaneous tablet of oestrogen.

The results with these two groups show conclusively that local stimulation by a foreign body in animals to which oestrogen is administered, does not produce in the thoracic serosa the same effect as in the abdominal cavity of the same animal.

Things change completely when there is, *besides* local stimuli, also a communication with the abdominal cavity. There was one animal in which there was a communication between the thoracic and abdominal cavities on the right and a metallic tablet on the left side. Several fibroids developed on both sides (Fig. 4). The metallic tablet on the left was also enveloped in a large tumour (Fig. 5); there was also a tumoural thickening of the thoracic scar.

SUMMARY.

The incidence of oestrogen-induced fibroids of the thoracic serosa in the guinea-pig was extremely small when compared with the incidence of similar fibroids of the abdominal serosa, uterine or extrauterine.

When an opening was cut in the diaphragm and a permanent communication established between the thoracic and abdominal cavities the incidence of

oestrogen-induced thoracic fibroids increased very considerably; there were thoracic fibroids in 7 out of 18 animals with such a communication.

In most of these animals part of the liver and of the intestine prolapsed to the thoracic cavity. But the mechanical stimulus due to this accident was seemingly not responsible for the increase of the incidence of oestrogen-induced thoracic fibroids. This was shown by the fact that in 24 animals without an abdominal-thoracic communication the capsule enveloping a foreign body introduced in the thoracic cavity (metallic tablet or a tablet of α-oestradiol) did not, or only exceptionally, become surrounded, under the influence of the oestrogen, by a fibroid as was the case with a similar foreign body (metallic tablet) introduced in the abdominal cavity of these animals.

The above experimental results suggest that the oestrogen-induced fibrous tumoral reaction of the peritoneum in the guinea-pig is mediated by an internal ambiental factor normally present in the abdominal serosal fluid, or a factor originating under the influence of the oestrogen and given up to the abdominal cavity. It is seemingly the absence of this mediating factor which protects the animals against production of fibroids in the thoracic cavity.

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THE ROLE OF INITIATING AND PROMOTING FACTORS IN THE PATHOGENESIS OF TUMOURS OF THE THYROID.

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It has been realized for a long time that two processes might be involved in the pathogenesis of neoplasms, one which is concerned with the transformation of normal into neoplastic cells, and the other which promotes the growth of neoplastic cells into a visible tumour. Friedewald and Rous (1944*a*, 1944*b*) used the term "initiating" for the former, and "promoting" for the latter process. Rous and Kidd (1941), and MacKenzie and Rous (1941) analyzing the effects of tar and carcinogenic hydrocarbons on the skin of rabbits, found that these agents "cause many more cells to become tumour cells than give rise to visible growths." Berenblum (1941*a*) discovered that the yield of tumours of the skin induced in mice by sub-optimal doses of carcinogenic hydrocarbons could be increased by subsequent application of croton oil, and used the term "epi-carcinogenic action" to describe this effect. Mottram (1944*a*) refined Berenblum's technique; he found that tumours could be obtained by a single application of benzpyrene if the painting with the carcinogen was followed by repeated application of croton oil. This has been confirmed by Berenblum and Shubik (1947*a*, 1947*b*).

It seemed important to investigate whether initiating and promoting factors were instrumental in the formation of tumours other than the skin. The thyroid was chosen as target organ and 2-acetylaminofluorene (A.A.F.) as the carcinogen. Bielschowsky (1945) obtained multiple adenomata of the thyroid by feeding A.A.F. to rats for 15 or 20 weeks, which was followed by administration of allyl-thiourea for up to 18 weeks. This experimental procedure did not allow a quantitative analysis of the factors involved in the pathogenesis of these thyroid tumours. Only by using small doses of A.A.F., which by itself are insufficient to induce neoplastic growth, could the role of initiating and promoting factors in the formation of multiple adenomata of the thyroid be ascertained.

METHODS.

The rats used were females, aged 6 weeks, belonging to a strain of Wistar rats which had been imported into New Zealand 18 years ago. No foreign stock has been added to the colony during this period. The A.A.F. was given by stomach tube in a watery suspension. Each rat received four or six doses of 2·5 mg. of the carcinogen during the week. 4-methyl 2-thiouracil was administered as a 0·01 per cent solution in the drinking water. The daily average consumption was 8 c.c. of this solution. The rats were fed a dry diet consisting of kibbled maize and wheat supplemented by a mixture of bran 30 per cent, pollard 25 per cent, bone meal 15 per cent, pea meal 15 per cent, and maize meal 15 per cent. Thyroxine was given in the form of thyroid siccum (*B.P.*, 1932). Three

1 grain tablets of this product were ground and mixed with 1 kg. of the dry pulverized diet.

The thyroids were dissected out at autopsy and fixed in formol-saline. Serial sections were cut at 7 μ and stained with Harris's haematoxylin and eosin.

EXPERIMENTAL.

Macroscopically the thyroids of the rats treated with A.A.F., and the glands of the controls receiving methyl thiouracil only, looked alike. In both groups the thyroid showed the typical picture of a hyperaemic goitre, the size of which increased progressively during the course of the experiment. However, the histological investigation of these glands revealed striking differences between the two groups (Fig. 1 and 2). As Table I shows, the rats in Group I*a*, which received 15 mg. of A.A.F. during the first week of the experiment, developed multiple adenomata. They were seen first in a rat killed 10 weeks after the withdrawal of the carcinogen, whereas an animal of the same group sacrificed 3 weeks earlier, showed only the well-known picture of hyperplasia and loss of colloid. The number and size of the adenomata increased during the course of the experiment, but their histological pattern remained essentially unaltered up to the twenty-first week, when the experiment was terminated. There was no indication of beginning malignancy, the nodules remaining sharply defined and showing orderly growth. Apart from the multiple adenomata of the thyroid, no other lesions were found which could be attributed to the action of A.A.F. Even when the duration of the experiment is prolonged for 18 months, the incidence of tumours induced by 10–15 mg. of A.A.F. in organs other than the stimulated thyroid is practically nil (to be published). The parathyroid glands were found to be rather large in all our rats receiving methyl thiouracil for prolonged periods. (This reaction of the parathyroid is under active investigation by Drs. W. E. Griesbach and J. Malcolm of the Medical School of Otago.)

Multiple adenomata were absent in the thyroids of all the controls (Table II), which were observed for 42 weeks. The only neoplastic changes seen in this group were single adenomata, the first of which was found in a rat which had received methyl thiouracil for 21 weeks. This was of minute size involving only a few follicles. In three of the four animals killed after 42 weeks of treatment with methyl thiouracil, single adenomata were also found. One of these was clearly recognizable at the post-mortem. Histologically it was a large cyst filled partly with blood, and partly with colloid; in the periphery of the cyst adenoma there was a zone of smaller follicles which were distinct from the follicles of the rest of the gland (Fig. 3). Here the epithelium had nuclei which were richer in chromatin, giving this area a bluish appearance in the stained section.

EXPLANATION OF PLATE.

FIG. 1.—Multiple adenomata (Rat 7, Group I*a*). × 11.

FIG. 2.—Simple hyperplasia (Rat 5, Group II). × 11.

FIG. 3.—Single adenoma (Rat 16, Group II). × 11.

FIG. 4.—Multiple cystic adenomata (Rat 10, Group I*b*). × 11.

FIG. 5.—Detail from Fig. 1. × 95.

FIG. 6.—Detail from Fig. 4. × 95.

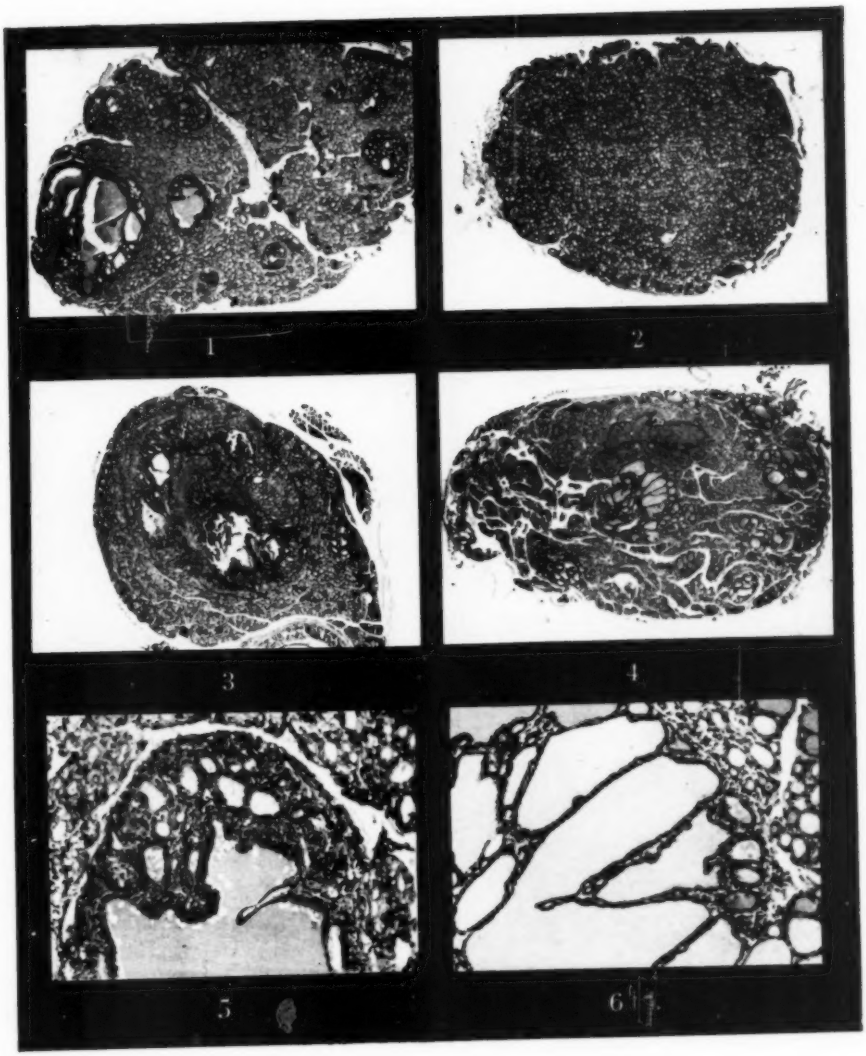
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TABLE I.—*Occurrence of Thyroid Adenomata in Rats Treated First with A.A.F. and Subsequently with Methyl Thiouracil.*

Group.		Rat No.	Weeks of stimulation (methyl thiouracil).	Thyroid.
Ia		1	8	Hyperplasia only.
		2	11	Multiple adenomata.
		3	15	,, ,,
		4	19	,, ,,
		5	21	,, ,,
		6	21	,, ,,
		7	21	,, ,,
Ib	4 weeks	8	25	,, ,,
	,,	9	25	,, ,,
Thyroxine treated;	5 weeks	10	26	,, ,,
	,,	11	26	,, ,,

TABLE II.—*Occurrence of Thyroid Adenomata in Rats Treated only with Methyl Thiouracil.*

Group.	Rat No.	Weeks of stimulation (methyl thiouracil).	Thyroid.
II (Controls)	1	8	Hyperplasia only.
	2	11	,, ,,
	3	15	,, ,,
	4	19	,, ,,
	5	21	,, ,,
	6	21	,, ,,
	7	21	Minute single adenoma.
	8	25	Hyperplasia only.
	9	25	,, ,,
	10	26	,, ,,
	11	26	,, ,,
	12	26	,, ,,
	13	42	,, ,,
	14	42	Single adenoma.
	15	42	,, ,,
	16	42	Large single cystic adenoma.

An investigation of the response of these multiple adenomata to the action of thyroxine, was undertaken at the suggestion of Dr. H. D. Purves of the Thyroid Research Department. Desiccated thyroid gland was given to 4 rats during the last 4 to 5 weeks of the experiment (Table I, Group I*b*), the administration of the methyl thiouracil being continued. Fig. 4 shows the effect

of the thyroid medication. The multiple adenomata persist, but show a pattern which differs considerably from the one seen in animals of Group I*a*. Whereas in the latter the epithelium of the hyperplastic tissue as well as of the adenomata is high and colloid cysts are only occasionally present (Fig. 5), the glands of the rats treated with desiccated thyroid show quite a different picture. Here the gland is full of colloid and the epithelium is low throughout; the adenomata frequently contain colloid cysts lined by low-cuboid or flat epithelium (Fig. 6). There is no indication that the administration of thyroxine in the dose employed had an adverse effect on the benign tumours of the thyroid. No signs of regression or necrosis were found.

Identical results were obtained in a second experiment. Eleven rats (Table III) received first methyl thiouracil for 2 weeks and subsequently the same treatment as the animals of Group I. Again desiccated thyroid was fed to rats No. 8–11. The first rat was killed in the eleventh week of the experiment, i.e. 8 weeks after the carcinogen was withdrawn. No adenomata were seen on histological examination. The other 10 rats, killed between the twelfth and twenty-sixth week, all had multiple adenomata. There was no difference in the structure of the thyroid glands between Groups I and III.

TABLE III.—*Occurrence of Thyroid Adenomata in Rats Treated First with Methyl Thiouracil for 2 Weeks, and Subsequently with A.A.F. and Methyl Thiouracil.*

Group.		Rat No.	Weeks of stimulation (methyl thiouracil).	Thyroid.	
III*a*		1	2 + 8	Hyperplasia only.	
		2	2 + 11	Multiple adenomata.	
		3	2 + 15	,,	,,
		4	2 + 19	,,	,,
		5	2 + 21	,,	,,
		6	2 + 21	,,	,,
		7	2 + 21	,,	,,
III*b*	4 weeks	8	2 + 25	,,	,,
Thyroxine treated;	,,	9	2 + 25	,,	,,
	5 weeks	10	2 + 26	,,	,,
	,,	11	2 + 26	,,	,,

It seemed important to establish whether the susceptibility of the thyroid gland to the action of A.A.F. could be increased by pre-treatment with a goitrogenic agent. Dr. F. Bielschowsky has allowed me to include in this paper an experiment performed by him in 1945 which he has already mentioned (Bielschowsky, 1947).

Twenty female rats received daily 6 mg. of allyl thiourea for 18 weeks, and subsequently a daily dose of 4 mg. of A.A.F. for 25 weeks. Rats No. 14 and 15 (Table IV) were killed a few days after the withdrawal of the carcinogen. The

remainder of the animals were killed when the presence of a tumour was suspected or when the animal appeared seriously ill.

TABLE IV.—*Occurrence of Thyroid Adenomata in Rats Treated with Allyl-Thiourea and Subsequently with A.A.F.*

Rat No.	Duration of experiment (days).	Thyroid.	Cancer in other organs.
1	469	Normal	None.
2	442	Large single adenoma	Small intestine.
3	461	Two adenomata	,, ,, uterus.
4	454	Normal	Liver, duct. acust. ext.
5	461	Single cystic adenoma	,, ,, ,, ,,
6	377	Normal	Liver.
7	356	Large single adenoma	Duct. acust. ext.
8	433	Normal	None.
9	433	,,	Liver.
10	426	,,	,,
11	377	Single adenoma	,,
12	468	Large single cystic adenoma	,,
13	400	Normal	Liver, small intestine
14	316	Single adenoma	None.
15	302	Normal	,,
16	412	,,	Duct. acust. ext.
17	384	Single cystic adenoma	,, ,, ,,
18	405	,, ,, ,,	Liver, lung (adenoma).
19	451	,, ,, ,,	Duct. acust. ext.
20	463	,, ,, ,,	Lung (adenoma).

The liver of all animals contained cystic cholangiomata.

Although the time of stimulation was long, and the carcinogen was fed for nearly half a year, there was not the slightest indication of an increased susceptibility of the thyroid to the action of A.A.F. (Table IV). The neoplastic changes found were single adenomata of varying size. These benign neoplasms were indistinguishable from the ones which appear after prolonged stimulation with goitrogenic agents, and which persist after their withdrawal. Only in one animal (rat 3) two minute nodules were present in the same lobe. It is of interest that A.A.F. did not transform these benign structures into cancers.

To test for the existence of latent neoplastic cells in the thyroid the following experiment was set up. Fourteen rats received first 4 doses of 2·5 mg. of A.A.F. given by stomach tube, and 4 to 18 weeks later treatment with methyl thiouracil was started. Table V shows the results. There was no essential difference whether or not the stimulation of the thyroid started immediately after pretreatment with the carcinogen or whether an interval elapsed before methyl thiouracil was given. All the animals which received methyl thiouracil for 13 or more weeks developed multiple adenomata, even when the interval was extended for 18 weeks.

TABLE V.—*Occurrence of Thyroid Adenomata in Rats which Received First* 10 *mg. of A.A.F. and Subsequently Methyl Thiouracil After an Interval of* 4–18 *Weeks.*

Rat No.	Interval (weeks).	Weeks of stimulation (methyl thiouracil).	Thyroid.
1	4	11	Multiple adenomata.
2	4	26	,, ,,
3	4	26	,, ,,
4	6	13	,, ,,
5	6	26	,, ,,
6	6	26	,, ,,
7	8	11	Hyperplasia only.
8	8	14	Multiple adenomata.
9	8	26	,, ,,
10	10	11	Hyperplasia only.
11	10	13	Multiple adenomata.
12	10	26	,, ,,
13	14	24	,, ,,
14	18	27	,, ,,

DISCUSSION.

The work of Rous and his collaborators, of Mottram, and of Berenblum, has firmly established the existence of latent neoplastic cells in the skin of animals treated with a dose of a carcinogen which in itself is ineffective to induce visible tumours. The existence of such latent cells in mice was demonstrated by applying an unspecific irritant to the skin which had received a single dose of a carcinogenic hydrocarbon (Mottram, 1944*a*; Berenblum and Shubik, 1947*a*). There is excellent agreement between the results of Mottram, and of Berenblum and Shubik except in one point. Mottram (1944*b*) believed that a hyperplastic epidermis is more susceptible to the action of the carcinogen than the normal one. Berenblum (1941*b*), and Berenblum and Shubik (1947*a*) could not find any evidence for a sensitizing action of croton oil applied previous to the carcinogen to the skin. The results obtained in this laboratory agree with those of Berenblum and Shubik. There was no evidence that pre-treatment of the thyroid with a goitrogenic agent sensitized the gland to the subsequent action of A.A.F.

It could be shown that a small dose of A.A.F., which alone is incapable of inducing visible tumours in any part of the body, must nevertheless transform many normal into neoplastic cells. No other explanation accounts for the formation of multiple adenomata of the thyroid which develop after 11 or more weeks of administration of methyl thiouracil. These neoplastic cells, from which the multiple adenomata of the thyroid originate, remain dormant unless an appropriate stimulus is applied to the tissue harbouring them.

The thyrotropic hormone of the pituitary is the active stimulus when methyl thiouracil, thiourea and other so-called goitrogenic agents are administered. In the experiments reported here, the thyrotropic hormone plays the role which croton oil plays in the experiments of Berenblum and Shubik (1947*a*, 1947*b*); A.A.F. in the doses used, acts on the thyroid in the same way as a single moderate does of a carcinogenic hydrocarbon. There exists, however, one difference between the action of croton oil and the effects obtained with prolonged hormonal stimulation. Croton oil, however long applied to the skin of mice, produces only diffuse hyperplasia, but several examples are known where long continued stimulation by hormones leads to formation of benign and malignant tumours. For instance, treatment with oestrogens leads to cancer of the breast in rats (Geschickter, 1942; Nelson, 1944), and administration of goitrogenic compounds induces cancer of the thyroid after 20 or more months (Purves and Griesbach, 1946), benign tumours appearing earlier (Purves and Griesbach, 1947). Similarly in spayed mice, tumours of the ovary can be obtained by transplantation of this organ into the spleen. Here the gonadotropic hormones are the stimulating agents (Biskind and Biskind, 1944). The tumours due to excessive hormonal stimulation develop rather slowly, and their pathogenesis must be different from that of the tumours described in this paper. The single adenomata which, as Bielschowsky (1945) has already shown, develop after stimulation with thyrotropic hormone in absence of a chemical carcinogen, very rarely appear as quickly and never with the regularity of the multiple tumours initiated by A.A.F. Even after 42 weeks some of the controls are free of neoplastic changes, whereas every single rat which had received previously A.A.F. developed multiple adenomata in a much shorter period. The multiple adenomata of the thyroid which we obtained, developed at a time when, as the controls show, hormonal stimulation alone produced mainly hyperplasia. They seem to appear nearly as quickly as the papillomata of the skin in the experiments of Mottram (1944*a*) and Berenblum and Shubik (1947*a*, 1947*b*). Unfortunately it is necessary to kill the animals in order to demonstrate the presence of the adenomata of the thyroid, so that it is difficult to discover the very early lesions.

It seems, therefore, justified to assume that, in the experiments reported in this paper, the thyrotropic hormone plays the same role in the pathogenesis of the multiple adenomata of the thyroid as the croton oil in the pathogenesis of the papillomata of the skin. The fact that only benign tumours are induced in both sets of experiments strengthen the analogy, and it seems legitimate to disregard the late effects of hormonal stimulation in the interpretation of the results. It is worth while mentioning that the choice of method is of utmost importance in experiments designed to demonstrate the role of initiating and promoting factors. For instance, when methylcholanthrene is used in high concentrations a single application is sufficient to induce benign or malignant tumours (Cramer and Stowell, 1943). Also large doses of goitrogenic agents, as used by Kuzell, Tripi, Gardner and Laqueur (1948), seem to hasten the appearance of multiple adenomata of the thyroid.

SUMMARY.

The induction of multiple adenomata of the thyroid by 10–15 mg. of acetylaminofluorene followed by administration of methyl thiouracil is described, confirming the results of Bielschowsky (1945) who used much larger doses of the

carcinogen and allyl thiourea as the goitrogenic agent. The experimental procedure used allows to distinguish between the initiating and promoting process in the formation of these tumours. The conceptions derived from the study of experimental cancer of the skin by Rous and collaborators, and by Berenblum and Shubik can be successfully applied to an analysis of the pathogenesis of experimental neoplasms of the thyroid.

I wish to thank Mr. T. H. Kennedy, of the Thyroid Research Department, for the sample of acetylaminofluorene synthesized by him, and Dr. F. Bielschowsky for his help in the preparation of this paper.

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SOME EFFECTS OF THIOL-COMPOUNDS ON TUMOUR INDUCTION BY CARCINOGENIC HYDROCARBONS.

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THE testing of substances for anticarcinogenic properties must proceed on purely empirical lines, or be based upon a hypothetical conception of the nature of some phase of the biochemical mechanism of chemical carcinogenesis.

Suggestive, but not conclusive, evidence has been obtained that S-metabolism is intimately connected with a primary stage in the interaction of chemical carcinogens with cellular constituents. A specific combination of a carcinogenic hydrocarbon with an enzyme-protein by way of –S–S– groups has been postulated by Wood and Fieser (1940), but no direct evidence in support of this possibility is available.

If SH-containing enzymes were involved in the essential metabolic changes which precede or accompany tumour induction, there are, theoretically, three ways of antagonizing such processes.

Firstly, the enzymes concerned could be rendered less active by the use of inhibitors of S-metabolism. This conception has been experimentally demonstrated by selecting several types of substances with this property in common, applying them to the skins of mice in conjunction with a carcinogen, and thereby checking the rate of tumour induction (Crabtree, 1940, 1941, 1944, 1945).

A second way of opposing carcinogenic action would depend upon the possibility of competition between carcinogens and structurally related non-carcinogens for the active centres of SH-containing enzymes which play a role in tumour induction. This conception prompted the work of Lacassagne, Buü-Hoi and Rudali (1945), who claimed to have shown that antagonisms of this type could be realized experimentally.

A third type of anticarcinogenic action could be envisaged, again arising from the possible association of S-metabolism and carcinogenic action. If, in addition to a carcinogen, a suitable SH-compound were presented to the cell, the latter might fix the carcinogen preferentially by a mechanism similar to that normally occurring between a carginogen and a specific cell-substrate. In this case the normal course of carcinogenic metabolism would be diverted, and the neoplastic change either retarded or prevented. This possibility is exemplified in the use of British anti-Lewisite (BAL), where combination of lewisite with SH-containing enzymes is minimized by a similar but more rapid combination with BAL.

This last conception has been put to the test in the experimental work recorded here.

MATERIALS AND METHODS.

The mice used were MRC/H hybrids, a robust type well suited for long experiments, fed on the standard balanced diet obtained commercially. Each experiment was carried out on 30 mice, of both sexes, except when toxic effects were anticipated, in which case 10 more were added.

In the groups receiving either the carcinogen alone or in conjunction with a substance which proved to be innocuous, the number of mice surviving the full course of the experiment was always 90 per cent or over. All mice were epilated over the scapular region once only, two days before the experiments began. The approximate amounts of carcinogen and tested substance used in any one experiment were as follows :

Solution used.	Period of application (weeks).	Weight of solute (mg.).
0·1% 3:4-benzpyrene . . .	20 .	1·0
0·2% methylcholanthrene . .	14 .	1·7
0·2% 1:2:5:6-dibenzanthracene .	28 .	2·8
2·0% S-compound . . .	25 .	250·0

The dissolved carcinogen was applied with a small brush (35–40 brushfuls per ml.) over an area of about 1 cm.^{2} on Mondays and Thursdays, and a solution of the substance tested with a larger brush (7–8 brushfuls per ml.) on Tuesdays, Wednesdays, Fridays and Saturdays over the same area but widely overlapping it. These treatments were continued until at least 90 per cent of the control mice carried tumours, when the applications of carcinogen were suspended, but the substance under test was further applied until tumours emerged in almost all the survivors. Except when testing 2:3-dimercapto-propanol (BAL), 3:4-benzpyrene was the only carcinogen used. A 0·1 per cent solution has been found most convenient, since with higher concentrations less obvious effects are produced by an inhibitor, and with lower concentrations the experiments are unduly prolonged, thus favouring any possible toxic action of the substance tested. With the strain of mice used, the average time for tumour induction with 0·1 per cent 3:4-benzpyrene applied twice weekly is $16{\cdot}0 \pm 0{\cdot}1$ weeks, a figure obtained repeatedly in many experiments.

The substances tested were chosen for no other reason than that of convenience. They include examples of monothiol and dithiol compounds, both aliphatic and aromatic, and also three fat-soluble derivatives of the amino-acids cysteine and cystine. It was presumed that the latter, prepared by the methods of Pirie (1931), would provide a local excess of cysteine in the skin, after hydrolysis and reduction.

RESULTS.

A representative selection of results is included in Table I. Many are omitted, being of doubtful significance due to the high percentage of mice dying without tumours. When this occurred in the early part of an experiment and clearly reflected the toxicity of the substance tested, the difficulty was easily met by using smaller doses of the toxic agent. But in some cases, and particularly when BAL was used, almost all the mice would survive in apparent good health for many weeks, but at the critical period for wart emergence would, without preliminary gross symptoms, die in numbers which made an assessment of the result impossible. Such an effect would be anticipated if BAL were excreted or destroyed more slowly than it was administered, but this delayed toxic action made it difficult to choose a dosage that was at the same time inhibitory towards tumour induction, and tolerated for several months by the

mice. In the experiments recorded in Table I this was largely achieved, though in other experiments, seemingly identical, the results were invalidated by this high middle-period death rate. In such cases the observed inhibitions appeared larger than in the experiments detailed above, but they were probably unreal, since they only applied to a selected residue of the more refractory mice.

TABLE I.—*Effect of Thiol-Compounds on the Rate of Induction of Tumours by Carcinogenic Hydrocarbons.*

Carcinogen.	Substances tested.	Solution applied.	Number of Mice. At start.	Dead without tumours.	With tumours.	Average induction time (weeks).
BP	..	..	30	1	29	16·0
„	Thiol-acetic acid	2% in ether	30	11	17	17·0
„	O-Thiol-benzoic acid	2% „	30	8	22	16·4
„	Thiophenol	2% „	30	4	24	16·5
„	Benzyl mercaptan	2% „	30	5	23	16·7
„	Acetylcysteine methyl ester	2% in acetone	30	2	27	16·1
„	Cysteine dimethyl ester	2% „	30	1	28	16·0
„	Diacetylcystine dimethyl ester	3% „	30	3	27	16·1
„	Toluene-3:4-dithiol	2% in ether	30	30	—	—
„	„ „ „	0·2% „	30	9	20	18·5
„	2:3-Dimercapto-propanol (BAL)	0·6% „	30	4	26	17·5
„	Ditto	1·5% „	30	5	25	19·0
„	„	2·0% „	36	7	27	21·8
„	„	3·0% „	40	12	27	22·5
„	„	6·0% „	30	30	—	—
MC	..	..	30	1	29	12·5
„	2:3-Dimercapto-propanol (BAL)	2·0% in ether	40	5	34	15·5
DBA	..	..	30	3	25	24·3
„	2:3-Dimercapto-propanol (BAL)	2·0% in ether	40	14	23	29·7

BP = 0·1 per cent 3:4-benzpyrene in ether + 2 per cent liquid paraffin (medicinal).
MC = 0·2 per cent methylcholanthrene in ether + 2 per cent liquid paraffin (medicinal).
DBA = 0·2 per cent 1:2:5:6-dibenzanthracene in acetone + 2 per cent liquid paraffin (medicinal).

Statistical Analysis of Table I by C. C. Spicer.

Influence of BAL on induction time.

The results for BAL have been analysed in two parts. Those for BAL and benzpyrene, where several percentages of BAL were used, can be analysed using the technique of regression (Fisher, 1947). That is to say a straight line is fitted to the mean induction times by the method of least squares; if the slope of this line is significantly different from zero, then BAL can be said to exert an inhibitory effect on the benzpyrene. The slope of the line is a measure of the efficacy of the BAL. It will be seen from Fig. 1 that a straight line gives a good description of the trend of induction time with increasing doses of BAL.

Allowance for the effect of animals dying before the appearance of a tumour (Lea, 1945) does not sensibly affect the conclusions, and the approach to normality of the observations is not improved by using a logarithmic time scale. For

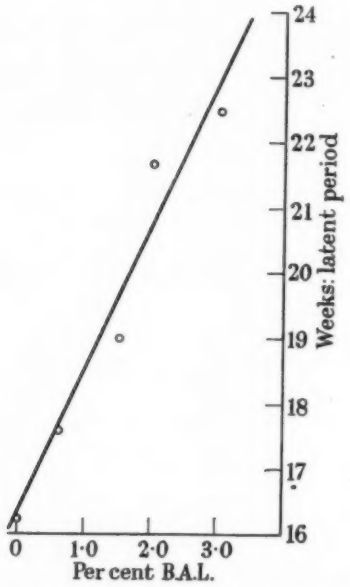

FIG. 1.—Graph showing relationship between average time of tumour induction by benzpyrene and per cent of BAL applied.

these reasons the analysis can be presented in its simplest form in a table of analysis of variance as follows :

	Degrees of freedom.	Sum of squares.	Mean squares.
Regression	1	36,066	36,066
Deviations from regression .	3	2,128	709
Between BAL percentages .	4	38,194	9,549
Within levels	128	63,685	498
Total	132	101,879	—

$F = 72$. 1 per cent point = 7.

The significance of the regression is beyond doubt, and since the deviations from the fitted line are not significantly large, it can be said that a straight line adequately fits the results. The equation of this line, which is plotted in the figure, is :

$$Y = 16{\cdot}27 + 2{\cdot}21\ X,$$

where Y is the induction time in weeks and X is the percentage of BAL used.

The results of using BAL with methylcholanthrene (MC) and dibenzanthracene (DBA) also show that a significant increase in the induction time has occurred.

In the case of MC the results can be analysed using Student's t-test (Fisher, 1947) thus :

Mean induction time MC alone	=	12·60 weeks
,, ,, ,, MC + BAL	=	15·38
Difference	=	2·78 weeks
Standard error of difference	=	0·553
$t_{(61)}$	=	5·03 (1% point $t = 2·7$)

which is highly significant. The corresponding results for DBA have been analysed in a slightly different way, since the variability of animals treated with BAL is nearly three times as great as that of the controls. The test used is that given by Fisher (1943).

In this case the increase in mean induction time was 5·54 weeks. From Fisher and Yates' tables it is found that any difference greater than 4·04 is significant at the 1 per cent point (using $n_1 = 22$, $s_1 = 1·28$, $n_2 = 24$, $s_2 = 0·72$).

Influence of mono-thiol compounds.

None of the individual results here show a significant increase in induction time after treatment. The data do, however, show some evidence that inhibition is occurring, since 7 out of 8 compounds show an increased latent period. If the treatments were entirely without effect it would be expected that the mean latent times would be scattered above and below that of the control with about equal frequency. On this basis the probability of obtaining the observed results is $8/2^8 = 0·03$, which is beyond the conventional level of significance at 0·05. The absolute value of the effect would, of course, be slight.

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Mono-thiols.

None of those tested affected the carcinogenic action of 3:4-benzpyrene. At the concentrations used no toxic effects supervened, except to a small extent when thiolacetic acid was used. The slight apparent inhibition in this case is probably a function of the relatively high death rate.

Di-thiols.

Toluene-3:4-dithiol, the organic reagent used for the determination of tin, is highly toxic in the mouse. Thirty mice received one brushful of a 2 per cent ethereal solution, about 2 mg.; 22 died overnight, and the rest over the next few days. A 0·2 per cent solution was tolerated when applied 4 times weekly, and caused a moderate retardation of the rate of tumour induction. Its toxicity makes it unsuitable for this type of experiment, and its great reactivity as a precipitant of proteins and a wide variety of metals precludes any concept of selective action.

BAL is the only substance tested which has produced unequivocal inhibitions (Fig. 2). Its effectiveness, within a limited range, increases with the concentration applied, but it lacks the potency of many other substances already studied, in particular those which lower the level of S-metabolism like bromobenzene, unsaturated dicarboxylic acids, and substances containing reactive halogen atoms (Crabtree, 1940, 1941, 1944, 1945; Berenblum, 1935).

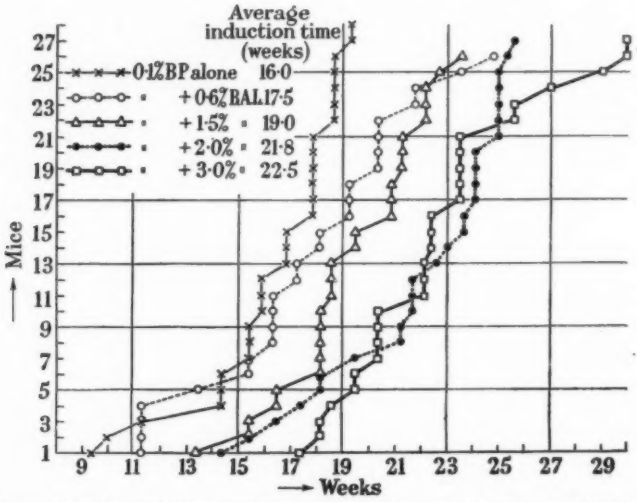

FIG. 2.—Influence of BAL, applied in various concentrations, on the rate of induction of tumours by 0·1 per cent 3:4-benzpyrene, showing details of results summarised in Table I.

The maximum doses tolerated by mice are many times greater, in proportion to body weight, than those producing toxic reactions in man during the treatment for arsenical dermatitis (Report, 1947). This may be partly due to differences in the mode of application, since intramuscular injection is commonly used clinically, but in painting experiments the fraction penetrating the skin is not known.

DISCUSSION.

The three types of competitive action mentioned in the introduction which might, in theory, oppose a carcinogenic process which involved sulphur at a primary stage have all, in some degree, been experimentally demonstrated. Limited success may well be due to the choice of inappropriate substances for testing, since ignorance precludes a rational prediction of potentially anti-carcinogenic reagents. The inadequate technique normally used certainly produces effects which are transient and intermittent, determined by the relative rates of elimination of the carcinogen and the inhibitor, and by such factors as their ease of absorption by the skin and their partition between aqueous and lipoidal phases of the tissues. The ideal substance would appear to be one whose physical characteristics closely parallel those of the carcinogen itself.

The most effective anticarcinogenic agents so far encountered are those containing reactive halogen atoms like mustard gas and acid chlorides, halogenbenzenes, and unsaturated dicarboxylic acids, all of which lower the activity of SH-enzymes or the effective concentrations of simpler SH-compounds like glutathione (Berenblum, 1935 ; Crabtree, 1940, 1941, 1944, 1945).

Apart from the present work little evidence is available that SH-compounds can influence carcinogenic action. The activities of the Staff of the Lankenau Hospital Research Institute, Philadelphia (1936), have been focused on a wide variety of biological effects produced by S-compounds, including studies concerned with the induction and growth of tumours. Partially oxidized derivatives of S-compounds have been used to retard growth, e.g. " a lesser mass increment " of transplanted tumours was observed when the hosts were injected with diphenylsulphoxide or diphennylsulphone (Hammett, 1930), and cystine disulphoxide proved inhibitory to the growth of spontaneous mouse tumours (Staff of the Lankenau Hospital, 1936). Conversely, compounds containing S in the reduced form, R.SH (the nature of R appears to be of secondary importance), were shown to stimulate cell-division in a wide variety of plant and animal tissues (Hammett, 1931). Arising from these results it was anticipated that the increased proliferation of the cells in mouse skin induced by treatment with an SH-compound would render the tissue more susceptible to the action of a carcinogen. p-thiocresol was therefore applied in conjunction with a carcinogen, but the opposite effect was observed, i.e. the SH-compound caused a pronounced retardation in the rate of tumour induction (Reimann and Hall, 1936).

Such inhibition stands in sharp contrast to the entirely innocuous behaviour towards carcinogenesis of the mono-thiol compounds used in the present work. The discrepancy can hardly be attributed to variations in the activities of the SH-compounds themselves, and must be referred to differences in experimental techniques. Reimann and Hall performed a variety of experiments differing only in the amounts of carcinogen and p-thiocresol administered, and the time-distribution of their application. The actual doses of carcinogen varied from experiment to experiment, e.g. the control group of mice received 2–4 times as much 1:2:5:6-dibenzanthracene over the latent period as the groups treated with *p*-thiocresol, and this feature alone would suffice to account for the wide variations in average induction times, and the apparent inhibitory effect. Moreover, the choice of lanoline as a solvent for *p*-thiocresol was unfortunate, since the work of Twort and Twort (1929), Rosicky and Hatschek (1943), Simpson, Carruthers and Cramer (1945), and Berenblum and Schoental (1947), have shown that lanoline greatly retards the carcinogenic action of tar, 3:4-benzpyrene, and methylcholanthrene. This added complicating factor augments the difficulty of accepting the experiments of Reimann and Hall as a demonstration of the anti-carcinogenic activity of SH-compounds.

The lack of activity of the mono-SH-compounds used in the present work, together with the fact that BAL, a di-SH-compound, can cause a moderate retardation of carcinogenic action, clearly signifies that the mere presentation of any SH-containing molecules (as implied by the Philadelphian workers) is not in itself sufficient to produce a biological effect ascribable to the SH-group. The experiences of Peters, Stocken and Thompson (1945) in the search for a suitable SH-compound, which culminated in the discovery of BAL, also emphasizes this. Apart from the question of variable toxicity, which rules out a substance like

toluene-3:4-dithiol, it is evident that the total molecule, with SH-groups appropriately disposed, is a factor of importance.

The analogy with the anti-vesicant activity of BAL cannot be pushed too far, since experiments *in vitro* clarified the nature of the reaction between BAL and lewisite, whereas any possible reaction between BAL and a carcinogen, modified in an unknown way, cannot be attempted at present.

Lacassagne, Buü-Hoi and Rudali (1945) have ostensibly shown that competition between a potent carcinogen and a structurally related substance with little or no carcinogenic activity can result in delayed tumour induction. Their work was an outcome of the conception that "the mutation which transforms a normal cell into a malignant cell is the result of the alteration of an organic substrate by the fixation to it of some toxic molecules (in this case polycyclic hydrocarbons)." Chrysene, 1:2:5:6-dibenzfluorene, five isomeric dimethyl-benzacridines and a trimethyl-benzacridine were chosen as near relations of methylcholanthrene, and 1:2:5:6-dibenzacridine for its structural resemblance to 1:2:5:6-dibenzanthracene. Though they state in general terms that inhibitions of carcinogenic action were demonstrated, the experimental details hardly justify so wide a conclusion, since only chrysene and 1:2:5:6-dibenzfluorene caused some slight delay in tumour emergence. Few mice were used, and some of the results are vitiated by high mortality rates; a large-scale repetition and extension of this type of experiment should clarify the potentialities of this method of antagonizing carcinogenic action.

No data are available as to the mechanism by which the tested compounds are eliminated by the mouse, but it is possible that sulphur plays a part in this; in which case any observed inhibitions could be attributed to local depletion of sulphur, and the compounds would then fall into the same category as naphthalene, anthracene and phenanthrene, which both lower the S-content of the skin and delay carcinogenesis (Crabtree, 1946).

The only known end-products of the metabolism of carcinogenic hydrocarbons are oxidized derivatives, but no proof has been obtained that any of the detoxication products has any intimate relation with the carcinogenic process. Similarly, only indirect indications are available that sulphur is important for cancer induction, and the manner of its possible intervention is purely hypothetical. The reactions initiating the changes which precede malignancy are not necessarily identical with those concerned with the elimination of carcinogens, though they may be closely related. The scheme of Weigert and Mottram (1946) envisages some intermediate stages in the transformation of 3:4-benzpyrene to 8-hydroxy-benzpyrene, and, if substantiated, may provide a link between the mode of excretion of oxidized products and the mechanisms by which sulphur intervenes in these metabolic transformations. However, the nature of the postulated cell-constituents which combine with the carcinogen through ethereal linkages remains unknown, and their significance, if any, in carcinogenesis is a matter for speculation.

SUMMARY.

1. As an extension of work based on the hypothesis that S-metabolism is concerned in the initial stages of carcinogenic action, a series of SH-containing substances have been applied to the skin of mice in conjunction with 3:4-benzpyrene, methylcholanthrene, or 1:2:5:6-dibenzanthracene.

2. All the monothiol compounds used were without effect on carcinogenesis. Of the dithiols tested, toluene-3:4-dithiol proved too toxic for this type of experiment, but definite anti-carcinogenic action was shown by 2:3-dimercapto-propanol (BAL), though less in degree than that induced by simple inhibitors of S-metabolism.

3. Some allied contributions from other workers are discussed in relation to these findings.

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NOTE ADDED TO PROOF.

In a paper just received (16.7.48) additional evidence is presented that BAL inhibits the induction of tumours in mice by 3:4-benzpyrene (Lusky, L. M., Braun, H. A., and Woodard, G.—(1947) *Cancer Res.*, **7**, 667.)

0·3 per cent of the carcinogen was painted on mouse skins twice weekly; 24 hours after each painting, 5 per cent BAL in an ointment base was applied. After 17 weeks the tumour incidence in the control mice was 85·4 per cent of the survivors, as against 57.5 per cent in the mice treated with BAL. The ointment base itself had no inhibitory action on tumour induction.

FURTHER EXPERIMENTS IN MICE WITH *p*-DIAZOAMINOBENZENE.

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OTSUKA (1935) claimed that rats and mice developed gastropapillomatosis when given *p*-diazoaminobenzene (DAAB) orally in a basal diet of unpolished rice (97 per cent), olive oil (2 per cent) and honey (1 per cent); the level of DAAB in this diet was 0·04 per cent. Sasaki (1935), in a general review, refers to papillomatous growths found in the stomachs of rats given DAAB orally and includes a photograph of a longitudinal section through one such stomach showing numerous papillomatous outgrowths from the squamous epithelium but no evidence of malignancy.

Hartwell (1941) reported that Shear and Stewart had obtained no tumours up to 17 months after repeated injections of crystals of DAAB subcutaneously into A strain mice.

The present author (1947) found that DAAB given orally to Wistar rats, whether in a good basal diet or in a vitamin A-deficient diet, would induce ulceropapillomas in the forestomach of an essentially similar structure to those obtained by many other workers with a wide variety of dietary deficiencies or abnormalities. DAAB seemed to interfere with vitamin-A storage and hence to cut down food consumption, leading to a deficiency of some other kind, such as that suggested by Zucker, Berg and Zucker (1945), which was the immediate cause of the forestomach lesions.

The present author (1947) was, however, unable to elicit such lesions in the stomachs of stock mice given DAAB orally, even up to 294 days, in either a balanced or one type of unbalanced diet. He concluded that Otsuka's diet (which was grossly deficient in several essentials) allowed a deficiency not existing in his own diets but necessary for eliciting such forestomach lesions in mice. He also found no tumours in similar mice injected subcutaneously with DAAB in arachis oil, on three occasions, up to 329 days after the first injection. DAAB thus appears not to be a carcinogen for the subcutaneous tissues of mice.

However, in another experiment which apparently broke fresh ground, the present author applied DAAB in acetone, in increasing strengths, to the nape of the neck of stock mice, and obtained two squamous carcinomas, one with spindle-cell metaplasia, in 2 of 8 mice surviving 346 days or more. This seemed to be sufficient grounds for classing DAAB as a carcinogen for the skin of mice.

A further series of experiments has now been carried out to check the previous findings with DAAB painted in acetone; at the same time, the effect, if any, of added croton oil was investigated. The results of these experiments are recorded below.

Experimental.

Stock mice, of mixed colours, were used, as before, in these experiments.

The *p*-diazoaminobenzene was obtained from British Drug Houses and purified as described (Kirby, 1947). The solvent was acetone (B.D.H. "analar") and a 5 per cent solution of DAAB was made up as required. For the experiments with added croton oil, DAAB was dissolved in a stock solution of 0·5 per cent croton oil in acetone. At the commencement, all mice were painted with the solution containing croton oil; after 15 days, the croton oil was withdrawn. Croton oil was re-introduced for one-third of the mice after a further 167 days, and the experiments therefore fall into two groups: (*a*) initial croton oil, and (*b*) initial-plus-subsequent croton oil.

Applications were made to the nape of the neck, after clipping away hair, thrice weekly for a total period of 545 days.

The basal diet throughout was rat-cake (Thomson, 1936).

Results.

(a) *Initial croton oil series.*—The survival rates of mice surviving more than 200 days are shown in Fig. 1. Four mice died between 80 and 130 days after

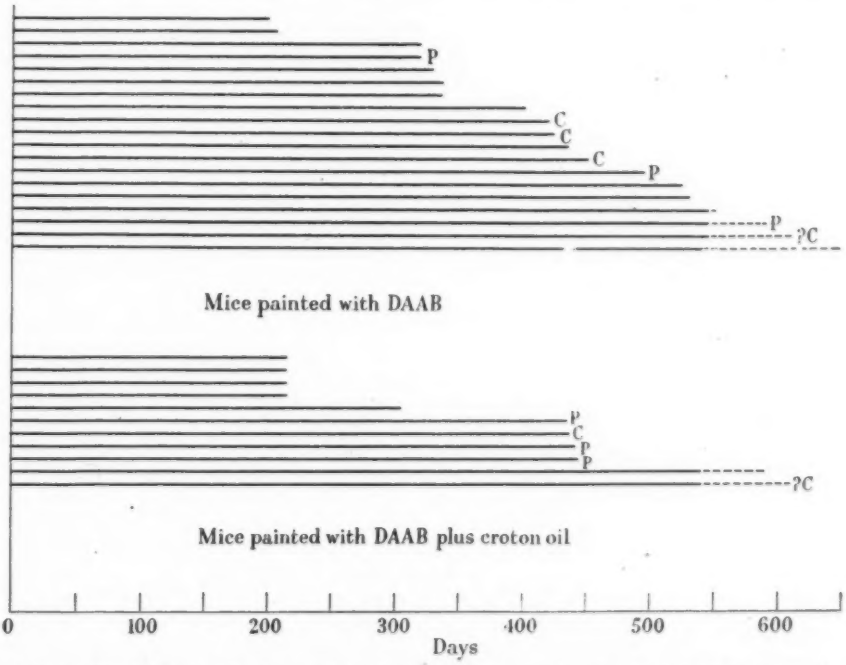


FIG. 1.—Survival rates of mice surviving beyond 200 days' painting with DAAB in acetone (*above*) or DAAB plus croton oil in acetone (*below*).
——— days painted.
– – – – days surviving after painting stopped.

the commencement of painting; of these, two showed acute yellow atrophy of the liver and one showed slight hyperkeratosis at the site of painting. Hyperkeratosis, with or without necrosis or ulceration, was common after 200 days, but the first papilloma was found in a mouse killed after 320 days' painting; this papilloma grew as a coxcomb-shaped wart. The liver of this mouse showed fatty change and focal necrosis. Two mice were killed after 422 and 424 days' painting respectively; the first had a circular, suppurating area at the site of painting (Fig. 2) about 1 inch diameter, which histological examination showed to be a squamous carcinoma (Fig. 3). The other mouse had a similar, though smaller, lesion which also proved to be a squamous carcinoma of low-grade malignancy. Although a mouse dying after 435 days showed no gross abnormality at the site of painting, another dying after 449 days had two tumours in the painted area; one of these was found to be a squamous carcinoma with invasion down to the depth of the muscle. A necrotic, warty outgrowth, showing parakeratosis beneath, and a multiple papillary growth, were found at the site of painting in mice painted for 495 and 545 days respectively, the latter dying at 588 days, i.e. 33 days after painting was stopped. Mice dying at 524, 528 and 549 days respectively showed nothing more than a little hyperkeratosis at the sites of painting. The last survivor and one of the mice from Group (*b*) unfortunately died at the same time (611 days) and were examined post-mortem in the absence of the author. One of these mice showed early squamous carcinoma, and the other simple infected ulceration; as there is no way of deciding which histological report belongs to which mouse, they will be excluded from consideration in comparing the results in the two groups. A mouse dying at 623 days showed no lesion at the site of injection.

Thus in 16 mice surviving more than 300 days' painting with 5 per cent DAAB in acetone, 3 developed simple papilloma, and 3 others developed squamous carcinoma, at the site of painting. Hyperkeratosis and/or ulceration was found in nearly all the rest.

Abdominal organs were only examined histologically when gross damage was visible, but the male dying at 449 days and one of the mice dying at 611 days showed amyloid changes in spleen, liver and kidney similar to those referred to in the previous paper (Kirby, 1947). The mouse dying at 623 days showed pulmonary adenoma.

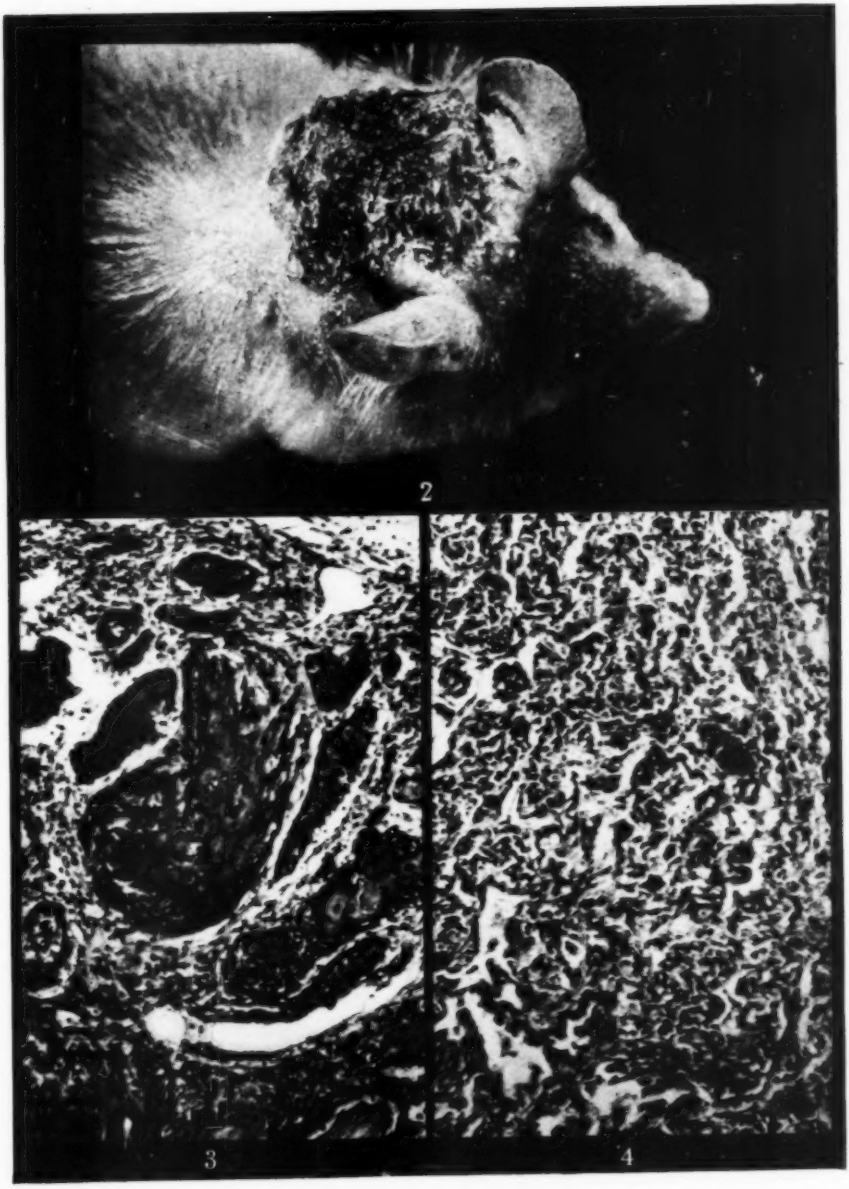
(b) *Initial-plus-subsequent croton oil series.*—Survival rates, beyond 200 days, are shown in Fig. 1. Only one mouse failed to survive 200 days. Only slight hyperkeratosis was found at the site of painting in 4 mice dying at 215 days and in 1 dying at 305 days. Of 3 mice dying at 435, 442 and 443 days respectively, all had papillomas, keratinized and partly necrotic, at the site of painting. A mouse, from the same box as the three just referred to, was killed at 437 days

EXPLANATION OF PLATES.

FIG. 2.—Squamous carcinoma at site of painting on a mouse painted for 442 days with 5 per cent DAAB in acetone.

FIG. 3.—Section through squamous carcinoma illustrated in Fig. 2; low-grade malignancy. × 110.

FIG. 4.—Adenocarcinoma in lung of mouse painted for 596 days with 5 per cent DAAB plus 0·5 per cent croton oil in acetone. × 110.



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with a large growth at the site of painting and this proved to be a squamous carcinoma. The last mouse in this box died after 590 days without gross lesion at the site of painting. As previously mentioned, the mouse dying at 611 days was confused with another belonging to Group (*a*). Necrosis was a frequent finding in spleens and livers of these mice, but amyloid change was not reported. The lungs of the mouse dying at 590 days showed multiple primary adenomas and one focus of adenocarcinoma (Fig. 4).

Thus of 6 mice (excluding the doubtful one) surviving 300 days' painting, 3 developed squamous papilloma, and one developed squamous carcinoma, at the site of painting. No other significant lesion was found in any mouse, save the pulmonary adenocarcinoma illustrated.

DISCUSSION.

The carcinogenic activity of DAAB, applied as a 5 per cent solution in acetone, for the skin of stock mice has been confirmed. Of 17 mice painted for more than 400 days, 5 developed squamous carcinoma, and 5 others squamous papilloma, at the site of painting, a tumour incidence of 60 per cent. The malignancy appears to have been low-grade in all cases, and metastases were never found. No evidence of spindle-cell metaplasia was seen in this series. As this substance is an intermediate in the manufacture of *p*-aminoazobenzene, the possible danger to workers handling it, especially in organic solvents, must not be overlooked.

Induction by DAAB of tumours at sites remote from that of application seems to be confined to the lung. One mouse in each group developed primary adenomas of this organ, and in one case adenocarcinoma was found as well; these tumours may have been spontaneous. No tumours were found in the liver of any mouse.

Co-carcinogen tests: Neither the incidence of skin carcinomas—3/11 (surviving 400 days) in Group (*a*) and 1/5 in Group (*b*)—nor the time of appearance—about 400 days in either Group—lends any weight to the view that croton oil acted as a co-carcinogen when applied concurrently with DAAB in Group (*b*). On the contrary, it would seem that croton oil had no effect either way on the induction of skin tumours. This suggests that the mechanism involved in carcinogenesis by DAAB differs from that of the polycyclic hydrocarbons. The question arises whether any initiating phase (Friedewald and Rous, 1944; Berenblum and Shubik, 1947) is involved at all, as, if it were, one would expect croton oil to increase the incidence of tumours in a given period. It is, of course, not known whether prolonged application of croton oil would yield more tumours after, say, 6 months' painting with DAAB in acetone than the painting with DAAB alone would elicit.

SUMMARY.

1. *p*-Diazoaminobenzene dissolved in acetone has been painted on the skin of stock mice for up to 545 days.

2. The carcinogenicity of this substance for mouse skin has been confirmed; tumour (benign and malignant) incidence was 60 per cent in mice painted for more than 400 days.

3. Croton oil painted concurrently caused no increase in the incidence of skin tumours, nor did it decrease the latent period; the significance of the absence of co-carcinogenic action of croton oil is discussed.

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THE ACTION OF 2-AMINOFLUORENE IN THREE PURE STRAINS OF MICE.

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THE carcinogenic activity of orally-administered 2-acetylaminofluorene, AAF, for a number of tissues, mainly epithelial, was first demonstrated by Wilson, DeEds and Cox (1941) for the rat, and subsequently confirmed for this species by Bielschowsky (1944, 1946) and others, for mice of various strains (Armstrong and Bonser, 1944, 1947; Foulds, 1947), for the fowl (Bielschowsky and Green, 1945) and for the cat (Harding, quoted by Bielschowsky, 1947). Recently Wilson, DeEds and Cox (1947*a*) have reported their own findings in mice of three pure strains. AAF has now been shown to be a carcinogen (when given *per os*) in eight strains of mice: Cba, IF, RIII, White Label, Strong A, C57 Black, C3H, and Bagg albino. Of the first five strains mentioned, Cba were most prone to both liver and bladder tumours; Strong A were the least susceptible (Armstrong and Bonser, 1947). A similar marked difference in susceptibility

has recently been shown for five strains of rats by Dunning, Curtis and Madsen (1947).

Wilson *et al.* (1941) were at that time unable to elicit tumours in rats by subcutaneous implantation of AAF, and, in view of the probable hydrolysis of AAF in the intestine to 2-aminofluorene, AF, they suggested that AAF might be converted to AF and that the latter product was the real carcinogen. It might be expected that AF would be more carcinogenic for rats than AAF in similar feeding experiments. However, in a recent paper, Wilson, DeEds and Cox (1947*b*) report tumours to have arisen more slowly and in fewer numbers in rats given AF orally than in similar rats receiving AAF in the same basal diet. They suggest that AF, being more soluble than AAF, may have been absorbed more rapidly and then excreted rapidly. AF derived from AAF by hydrolysis in the intestine could only be absorbed as fast as it was liberated. However, against this view it must be said that AF, once absorbed, should be excreted as fast in either case and that tissues in remote sites will stand the same chance of receiving AF, whether AF or AAF be given orally, provided the presentation is continuous. It seems possible that the hydrolysis takes place after absorption; studies on the liver after ingestion of AAF, using the extraction technique of Miller and Baumann (1945) and the estimation method of Westfall (1945), should yield information to test this point. It is significant that Wilson, DeEds and Cox (1947*a*) report tumours and other changes after implantation of crystalline AAF for long periods.

Bielschowsky (1944) painted AF in acetone thrice weekly on the epilated skin of Wistar rats. Contrary to expectation, no tumours at the site of painting occurred, but all 5 rats developed liver tumour by 280 days and one had a tumour of the *ductus acusticus externus*. The carcinogenic activity of AF upon distant sites was clearly established; Harris (1947) has also obtained similar results. Wilson *et al.* (1947*b*) report liver tumours and carcinoma of the bladder and kidney in 5 mice of the C57 Black strain given AF orally at a level of 0·25 per cent.

Experiments were begun in 1944 in this department to test the action of AF in three pure strains of mice, C3H, Cba and C57 Black, when presented by either of two routes, skin painting or subcutaneous injection. The results of these experiments are set out below.

Experimental.

2-Aminofluorene was prepared from fluorene by nitration and reduction according to the method of Diels (1908). After recrystallization from ethanol and from benzene, the product was still cream-coloured.

The mice used in this experiment were all bred in the Department; they were kept in metal boxes with screen bottoms. The basal diet throughout was rat-cake nuts (Thomson, 1936) and water was given *ad lib.*

Painting experiments.—A 4 per cent solution of AF in acetone (B.D.H. "analar") was applied twice weekly by means of a glass rod to the nape of the neck after clipping away the hair. The solution of AF turned brown in a few weeks and was therefore renewed at frequent intervals.

In order to enhance the effect, if any, of AF at the site of application, 0·5 per cent of croton oil was incorporated in the acetone used for making up the AF solutions.

Injection experiments.

2 per cent solutions of AF were used for subcutaneous injection. At first, arachis oil was used as vehicle, but later, tricaprylin became available and was used in place of arachis oil. Injections were made at intervals of approximately 4 weeks, in alternate flanks; 0·5 ml. was injected on each occasion.

Results.

Painting experiments.

(*a*) *C57 Black mice.*—No tumours were found at the site of painting; nor were tumours of the bladder, kidney or mammary gland evoked. Only one mouse, a male killed at 402 days, showed hepatomatous change in the liver, and this was of a diffuse nature. This mouse also showed leukaemic infiltration of the liver and spleen, a condition seen in four other mice dying between 226 and 466 days. No other frequent lesion was seen in this group of mice. Three mice survived 541, 546 and 597 days respectively.

(*b*) *Cba mice.*—Atrophic change, hyperkeratosis and ulceration were found at the site of painting in some of these mice; one mouse, a female killed at 530 days, had an invasive, well-differentiated squamous carcinoma. The kidneys were free of tumours, but one mouse, a female killed at 582 days, had a well-differentiated squamous carcinoma of the bladder, and another, a male dying at 81 days, showed moderate hyperplasia of the bladder epithelium. Seventeen mice died between 17 and 125 days after painting was begun; 11 of these mice showed necrosis of the liver, often with hypertrophy of remaining areas of parenchyma. The male dying at 81 days had hepatoma consisting of narrow columns of cells in a highly vascular sinusoidal network. A female dying at 351 days showed no abnormality, but of 7 mice dying between 530 and 594 days, all showed one or more liver tumours, some solid, some diffuse, with three cases being notably vascular. Six of these seven mice were females, the other mouse being the only male to survive more than 125 days. One female, killed at 582 days, showed a mammary adenocarcinoma, and another, killed the same day, had a granulosa-cell tumour of the ovary.

(*c*) *C3H mice.*—Thirteen mice survived 52 to 126 days; the rest survived 206 days or more. Three females, killed at 206, 235 and 235 days respectively, had one or two mammary adenocarcinomas. A fourth female was killed at 242 days without any gross tumour. Three males survived 409, 426 and 506 days respectively; the first and the last of these showed hepatoma; the other had primary renal carcinoma. The bladders of the C3H mice showed no hyperplastic lesion, and the sites of painting showed no more than atrophic change.

Injection experiments.

(*a*) *C57 Black mice.*—In almost all mice only a minimal tissue reaction occurred at the site of injection; a male, injected in the right flank five times with the arachis oil solution, and once with the tricaprylin solution, was killed at 406 days with a large sarcoma in that flank. This mouse, which also received five injections in the left flank, had a tumour in the liver which proved to be a hepatoma. Hepatoma was also found in females injected seven times and dying

TABLE I.—*Incidence of Tumours in Mice Painted with AF in Acetone.*

	C57 Black			Cba			C3H.		
	M.	F.	Total.	M.	F.	Total.	M.	F.	Total.
Mice examined post-mortem	13	3	16	16	10	26	12	8	20
Site of painting	0	0	0	0	1	1	0	0	0
Liver	1	0	1	2	6	8	2	0	2
Mammary gland	0	0	0	0	1	1	0	3	3
Ovary	0	0	0	0	1	1	0	0	0
Kidney	0	0	0	0	0	0	1	0	1
Bladder	0	0	0	0	1	1	0	0	0

TABLE II.—*Incidence of Tumours in Mice Injected Subcutaneously with AF in Oil.*

	C57 Black.			Cba.			C3H.		
	M.	F.	Total.	M.	F.	Total.	M.	F.	Total.
Mice examined post-mortem	15	9	24	19	10	29	23	12	35
Site of injection	1	0	1	0	0	0	0	0	0
Liver	1	4	5	3	2	5	1	0	1
Mammary gland	0	0	0	0	0	0	0	1	1
Ovary	0	0	0	0	1	1	0	0	0

at 147 and 152 days respectively, in a female injected ten times and killed at 328 days, and in another female injected 11 times and dying at 498 days. Ten male and two female mice survived over 500 days, four of these dying between 602 and 616 days; extensive leukaemic infiltration was seen in two of these mice, but no liver tumours (or tumours in other sites) were found in any. Kidney and bladder tumours were not found, nor were mammary tumours.

(*b*) *Cba mice.*—Only cysts with minimal tissue reaction were found at the sites of injection in these mice. A variety of degenerative changes were found in the liver in most mice; a male, injected eight times and dying at 285 days, had hepatoma at the edge of the left lobe. Hepatomas were also found in three mice injected 11 times; in two males dying at 565 and 574 days respectively, and in a female dying at 630 days. A cystic cholangio-hepatoma was present in another female dying at 622 days. Mammary tumours were not found, but a female dying at 468 days had a folliculoid, granulosa-cell tumour of the ovary. No tumours were found in the bladder or kidney in any of these mice.

(*c*) *C3H mice.*—No tumours were found at the sites of injection, only minimal tissue reaction. Nor were tumours present in the ovary, kidney or bladder. Only one mouse, a male injected nine times (arachis oil solution only) and dying at 242 days, had hepatoma. All mice succumbed by 259 days except one female which was killed at 391 days with a mammary adenocarcinoma.

DISCUSSION.

AF proved to have very low activity, if any, as a local carcinogen or sarcogen, one Cba mouse having a squamous carcinoma at the site of painting after 530 days, and one C57 black mouse having a sarcoma in the right flank at 406 days. The long period required in each case means that AF is a relatively weak carcinogen. Croton oil seems to have had no effect; this does not necessarily prove that AF is non-carcinogenic, as croton oil failed to enhance the activity of *p*-diazoaminobenzene which is now known to be carcinogenic for mouse skin (Kirby,

1948). The negative findings of Wilson, DeEds and Cox (1947*b*) who implanted crystals of AF subcutaneously in rats, and of Bielschowsky (1944) who painted AF in acetone on the skin of rats, seem to indicate that AF is not a *local* carcinogen in this species either.

Liver tumours appear to have been induced in all three species. In C57 Black mice painted, only one hepatoma was found among 15 mice surviving

TABLE III.—*Incidence, According to Age, of Liver Tumours in Mice Receiving AF by Skin Painting or Subcutaneous Injection.*

Route.	Strain.	Sex.	Time in days (≥). 150	200	250	300	350	400	450	500	550	600	650	Total.
Skin painting.	C57	M	0/2	0/0	0/1	0/0	0/0	0/3	1/2	0/1	0/2	0/2	—	1/13
		F	0/0	0/0	0/0	0/2	0/0	0/0	0/1	—	—	—	—	0/3
	Cba	M	1/15	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1	—	2/16
		F	0/3	0/0	0/0	0/0	0/0	0/1	0/0	0/0	1/1	5/5	—	6/10
	C3H	M	0/9	0/0	0/0	0/0	0/0	0/0	1/2	0/0	1/1	—	—	2/12
		F	0/4	0/0	0/4	—	—	—	—	—	—	—	—	0/8
Subcutaneous injection.	C57	M	0/0	0/0	0/0	0/1	0/0	0/2	1/2	0/0	0/3	0/3	0/4	1/15
		F	1/1	1/1	0/1	0/0	1/1	0/1	0/0	1/2	0/1	0/1	0/0	4/9
	Cba	M	0/9	0/2	0/4	1/1	0/0	0/0	0/0	0/0	0/0	2/3	—	3/19
		F	0/3	0/3	0/0	0/0	0/0	0/1	0/0	0/1	0/0	0/0	2/2	2/10
	C3H	M	0/9	0/1	1/7	0/6	—	—	—	—	—	—	—	1/23
		F	0/9	0/0	0/2	0/0	0/0	0/1	—	—	—	—	—	0/12

more than 200 days (see Table III), and this was found in a male killed at 402 days. Among mice of this strain injected subcutaneously, hepatoma was found in one male, killed at 406 days, out of 15 males surviving more than 250 days ; one female out of five surviving more than 400 days, had a hepatoma at 498 days. All these hepatomas arose between 400 and 500 days ; in our experience, spontaneous hepatoma may arise in this strain as early as 456 days (Kirby, 1945). Hence, the hepatomas in the above three mice may have been spontaneous. However, two other female C57 Black mice in the injected group developed hepatoma by 147 and 152 days respectively, and a third, killed at 328 days, showed diffuse hepatomatous change as well as leukæmic infiltration. These three cases were probably due to the action of AF which seems to be a carcinogen for the liver of C57 Black mice, at least of the female sex.

Wilson *et al.* (1947*b*) fed C57 Black mice with AF at various levels in the diet ; of five mice receiving the 0·25 per cent level, " 2 showed distinct adenoma formation in the liver," but the period required is not stated.

The incidence of liver tumours among Cba mice was higher than that in either of the other two strains (Table III). But 10 of the 13 mice so affected died at 21–22 months of age after between 565 and 630 days' treatment. Another of the painted females showed hepatoma when it died at 20 months after 530 days' treatment. According to Pybus and Miller (1942), the incidence of spontaneous hepatoma in Cba mice over 13 months old is 40·7 per cent in males and 26·6 per cent in females, with the earliest tumour in males at 19 months, but at 13 months in females, although the mode for both sexes is 30 months. The probability that the hepatomas in all 11 mice in the Cba groups were spontaneous is therefore very great, and these tumours cannot be ascribed to the action of AF. However, one painted male dying at 81 days had a highly vascular hepatoma and one male

of the injected group had hepatoma at the edge of the left lobe of the liver when it died at 285 days. These last two cases seem to be too early to be spontaneous and so AF may be regarded as weakly carcinogenic for Cba mouse liver also, at least in males, when either painted on the skin or injected subcutaneously.

Mice in the C3H groups did not survive as long as those in the other two groups, only one injected mouse living more than 300 days. No females developed liver tumours, but only one survived more than 250 days. One injected male of this strain had hepatomas by 242 days; two males of the painted group had hepatomas by 409 and 506 days respectively. Burns and Schenken (1943) record a spontaneous primary hepatoma in a C3H male 14·5 months (440 days) old. Thus the mouse dying at 506 days may well have borne spontaneous tumours, and this possibility may also apply to that dying at 409 days. The earliest case seems to be undoubtedly induced by AF which is therefore probably carcinogenic for the liver when injected subcutaneously in male C3H mice.

Among 12 Cba mice given 2-acetylaminofluorene (AAF) orally by Armstrong and Bonser (1944), which survived more than 12 weeks, four showed liver tumours, but two were females dying at 63 and 65 weeks respectively and therefore liable to bear spontaneous tumours. Armstrong and Bonser (1947) record three malignant hepatomas in seven male mice and five benign hepatomas in eight females given AAF orally. The time required for these tumours to develop is not recorded, but the incidence is well above that for spontaneous hepatoma in this species and, moreover, the latter always appear to remain benign. Hence AAF and AF both appear to be carcinogenic for the liver of Cba mice; AF is slightly active also in C3H and C57 Black mice.

Armstrong and Bonser (1944, 1947) record a high incidence of bladder tumours, mostly malignant, in Cba mice given AAF orally. Wilson *et al.* (1947*b*) found carcinoma in the bladders of three of five C57 Black mice given AF orally. In the present series no bladder tumours were found in mice injected subcutaneously with AF and only in one female Cba mouse, painted with AF and killed at 582 days. Thus AF seems to be a bladder carcinogen when given orally to C57 Black mice, but not when painted or injected subcutaneously. C3H mice appear to be unaffected in this organ.

The kidney was free from tumour in all three strains after injection of AF. Painted mice of the C57 Black and Cba strains likewise had no kidney tumours, but a probable renal carcinoma was found in a painted C3H mouse, a male dying at 426 days. Wilson *et al.* (1947*b*) record a carcinoma of the kidney in one of their C57 Black mice given AF orally. Armstrong and Bonser do not record any kidney tumour in Cba mice given AAF orally. Neither compound is very active for mouse kidney.

Armstrong and Bonser (1947) record one mammary cancer in 9 females given AAF orally and surviving more than 20 weeks. One Cba female in the present series, painted with AF, showed mammary adenocarcinoma at 582 days. Spontaneous mammary tumours in this strain seem not to occur and the solitary tumour found in each series referred to above is therefore considered to be significant. No mammary gland tumours were found in C57 Black mice painted or injected with AF; the four mammary tumours in C3H females were almost certainly spontaneous.

The only other tumours found in the three strains painted or injected subcutaneously with AF were granulosa-cell tumours of the ovary in two Cba females.

AF thus appears to be a weak carcinogen for mice of C57 Black, Cba and C3H strains. Of these, Cba appears to be the most susceptible, but even so the incidence of liver and bladder tumours is much below that following oral administration of AAF.

SUMMARY.

1. 2-Aminofluorene has been painted in acetone solution on the skin of mice of the C57 Black, Cba and C3H strains, and injected subcutaneously in oil into similar mice of these three strains.

2. Croton oil was incorporated in the solution used for skin painting, but its effect, if any, must have been slight, as only one tumour appeared at the site of painting in a total of 28 mice surviving more than 200 days' treatment.

3. A low incidence of tumours was also found at the site of injection, in the liver, bladder, kidney, mammary gland and ovary.

4. Of the three strains used, the Cba strain seemed most susceptible.

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THE METABOLISM OF 3:4-BENZPYRENE IN HUMAN BEINGS AFTER INTRAVENOUS INJECTION.

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EARLIER investigations by Weigert and Mottram (1946) have shown that 3:4-benzpyrene after administration to the animal organism is metabolized to at least four different derivatives, labelled by Weigert and Mottram as BPX_1, BPX_2, BPF_1 BPF_2. In a later investigation Weigert, Calcutt and Powell showed that after application of 3:4-benzypyrene to the skin of mice only the presence of BPX_2 was detected.

That a similarity exists between the metabolism of 3:4-benzpyrene in animals and human beings (patients suffering from leukaemia) has already been shown (Iversen, 1947).

The following investigation shows that the intravenous injection of 3:4-benzpyrene in human beings has an effect upon the amount of nitrogen in plasma, and that only—or mainly—the BPX_1 derivatives are found in plasma.

MATERIAL AND METHODS.

One part of 3:4-benzpyrene was fused with four parts of Postonal (a polymerization product of ethylenoxide), and carbon-dioxide free distilled water was added to the melted mixture, which was then shaken vigorously. This emulsion was stable for 3–4 hours. The diameter of the particles of the emulsion was between 6 to 15μ.

Immediately after the emulsion was prepared it was injected intravenously in patients suffering from myeloid or lymphatic leukaemia. The doses used were 300, 500 and 800 mg. 3:4-benzpyrene in 30, 40 and 80 c.c. emulsion.

Blood samples (one part 3·8 per cent sodium-citrate solution plus 9 parts blood) were taken from a cubital vein immediately before, immediately after and at various times after the injection. The refractive index determined in sodium light with an Abbe's refractometer and the content of nitrogen by means of Kjeldahl analysis were determined in plasma obtained after centrifuging the blood samples for half an hour at 2000 r.p.m.

Five c.c. ethanol was added to 2 c.c. plasma, and after centrifuging for 15 minutes at 2000 r.p.m., the alcoholic solution was poured into measure glasses and the volume made up to 7 c.c. with ethanol, whereafter the absorption of the alcoholic solution was measured on a Beckmann photo-electric spectrophotometer.

After the precipitate was dried to constant weight the amount of nitrogen in the precipitate was determined by Kjeldahl analysis.

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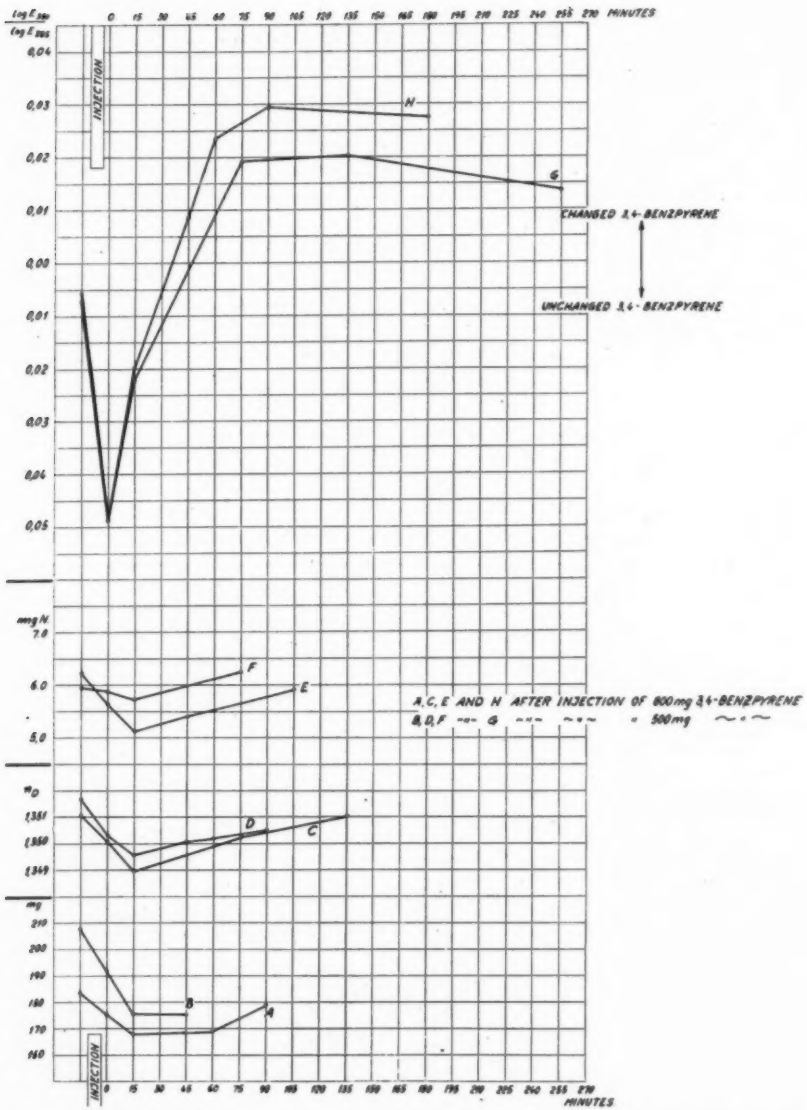

FIG. 1.—A and B show the amount in mg. of dried precipitate in plasma from different blood samples obtained at various times before and after the injection. C and D show the variation of the refractive index of plasma in relation to the injection. E and F show the amount of nitrogen in (475 mm.^{3}) plasma.

Fig. 1, curves A and B, shows a distinct decrease in the weight of precipitate which is most conspicuous at approximately 15 minutes after the injection.

In curves C and D also the decrease is greatest at approximately 15 minutes after the injection.

Curves E and F show the same fluctuations as in A, B, C and D.

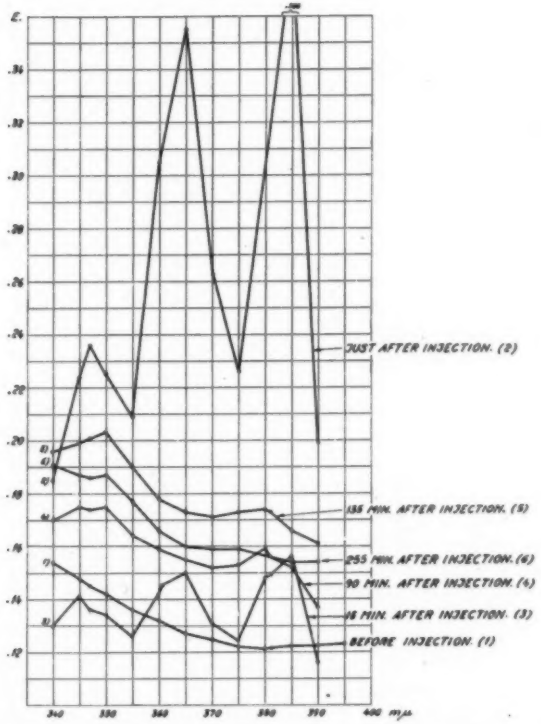

FIG. 2.—Absorption curves of plasma extracted with ethanol before and after intravenous injection of 3:4-benzpyrene.

In order to make sure that the emulsifier (Postonal) itself had no effect on the amount of nitrogen in plasma, 8 g. of Postonal, which is more than twice the amount ordinarily used for the benzpyrene emulsions, were injected intravenously into a patient. No changes in the plasma as described above were noticed.

Fig. 2 shows that the maxima of absorption characteristic of 3:4-benzpyrene are, little by little, altered to maxima of absorption characteristic of the metabolites labelled as BPX_1. The differences in position on the ordinate axis are seen to correspond with the fluctuations in the amount of nitrogen in plasma, which may mean that the fraction of alcohol-soluble substances varies correspondingly.

The results of Kjeldahl analysis on the precipitates showed that there was no noticeable difference between the percentage of nitrogen in the different samples of precipitate, which may mean that the composition of protein in plasma does not alter considerably in relation to an intravenous injection of 3:4-benzpyrene, or that it varies so little that it has been impossible, with the technique used, to determine the difference.

It would be possible to obtain a quantitative estimation of the proportion between the amount of unchanged benzpyrene and changed benzpyrene, and thus get an expression for the speed with which the metabolism (or detoxication ?) of 3:4-benzpyrene takes place by determining the proportions between the extinction at 380 mμ. (maximum for X_1 derivative) and at 385 mμ. (maximum for 3:4-benzpyrene).

The quotient $\frac{\text{E } 380 \text{ m}\mu.}{\text{E } 385 \text{ m}\mu.}$ for benzpyrene in 10 single determinations was shown to be 0·8599. The same quotient for the X_1 derivative is unknown and could not be determined, as it was not possible to obtain the X_1 derivative in a pure form, but the quotient must be higher than 1·00, as the absorption of the X_1 derivative is more intense at 380 mμ. than at 385 mμ.

The quotients obtained from alcohol-extracted plasma at different times before and after the injection are shown in Fig. 1, curves G and H. The curves show that the concentration of unchanged benzpyrene rapidly decreases, whereas the concentration of changed benzpyrene (X_1 derivative) rapidly increases. The curves further show that the time at which the rate of conversion is highest nearly coincides with the time at which the decrease in nitrogen-(protein-) content of the plasma is most conspicuous. Additionally the curves indicate that after the maximum concentration of changed hydrocarbon has been reached, elimination takes place at a constant speed.

That the conversion may take place in the liver after an intravenous injection is an obvious consideration, and the following experiment would indicate that this is the case in rabbits:

Blood is transfused through a living rabbit liver (Nielsen, 1933), and to the circulating blood is added 10 mg. 3:4-benzpyrene emulsified in the usual way; samples of blood are taken before and at various times after the injection. Fig. 3 shows, as in the case of human beings, that the absorption maxima of 3:4-benzpyrene are altered gradually to the maxima of absorption characteristic of the X_1 derivatives. That these curves appear higher and higher on the ordinate axis may be accounted for by the fact that it is not possible for the system to excrete. The variations in the quotient 380/385 mμ. from two such experiments with rabbits are seen in Fig. 4, curves D and E. The quotients for the alcohol extracted livers were 1·10 and 1·02. The fact that these quotients are higher than in the sera strongly supports the theory that the conversion of the hydrocarbon takes place in the liver.

Fig. 4, curve C, shows the variations of the quotient 380/385 mμ. for alcohol-extracted plasma from a patient suffering from lymphatic leukaemia. This curve has a noticeably different course from the quotient curve obtained from myeloid leukaemia, which may mean a slower rate of conversion, in the case of lymphatic leukaemia. Whether this difference in the rate of conversion of 3:4-benzpyrene is due to differences in the individuals themselves, to differences in the amount of benzpyrene injected, or to a difference between myeloid and

lymphatic leukaemia, it is not yet possible to decide, since the material on lymphatic leukaemia has been too limited to permit definite conclusions. Fig. 4, curve C, indicates clearly that the conversion takes place at a markedly lower speed than in the other instances mentioned. In this connection it is an interesting phenomenon that it seems easier to influence a lymphatic leukaemia by

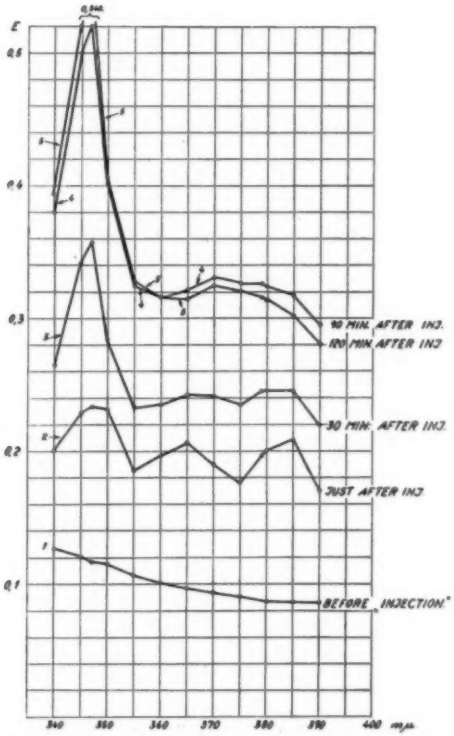

FIG. 3.—Absorption curves of the alcohol-extracted rabbit sera. (Defibrinated blood was used in the system.)

intravenous injections of polycyclic aromatic hydrocarbons than to influence a myeloid leukaemia (J. Engelbreth-Holm, private communication).

There are some observations which indicate that the metabolism of 3:4-benzpyrene is accompanied by changes in the metabolism of the protein. Thus it is a common reaction in all patients, after intravenous injection of benzpyrene (or other carcinogenic hydrocarbons), to get an attack of high temperature (40–41° C.), which appears approximately 1–2 hours after the injection and lasts for 4–6 hours. One of the patients, after an injection of 500 mg. benzpyrene, preceded by four earlier injections, sustained a severe attack of (protein ?) shock, which appeared 4–5 hours after the injection and lasted for 2–3 hours. It ought

perhaps to be mentioned in this connection that the patients during, or immediately after, but never later than half an hour after the injection, sometimes got slight attacks of pulmonary infarcts.

Whether one considers the changes in the amount of nitrogen (protein) in plasma as attached to the metabolism of 3:4-benzpyrene or as bearing no relation

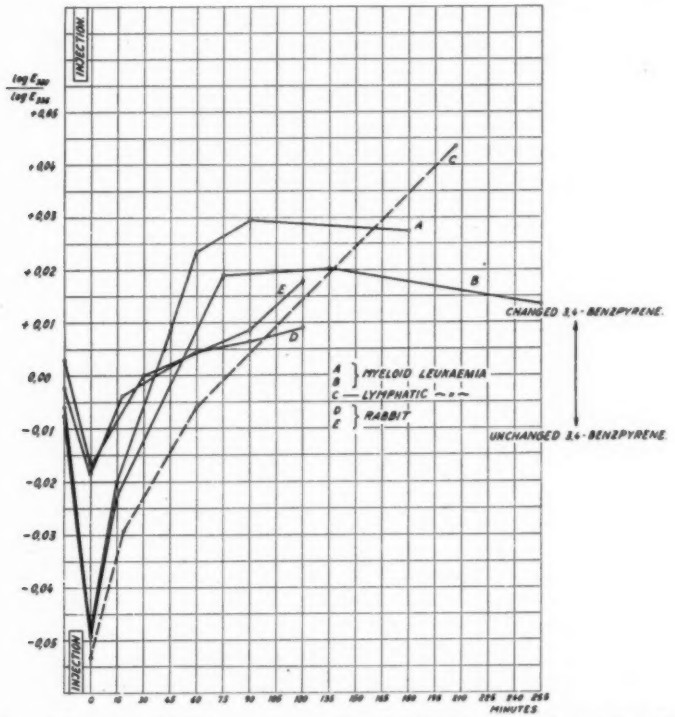

FIG. 4.—A and B = Myeloid leukaemia. C = Lymphatic leukaemia. D and E = Rabbit.

to the conversion of 3:4-benzpyrene, but accompanying the intravenous injection of 3:4-benzpyrene in an apparently regular way, it is possible, if one considers the concentration of unchanged benzpyrene as being highest in the blood sample obtained immediately after the injection, to calculate the relative amounts of X_1 derivative of the different blood samples by using the following equation:

$$\frac{\text{conc. } X}{\text{conc. } Y} = \frac{\left(A_{L_0} - {}^{k}_{L_0}\right) - \left(\begin{matrix}{}^{a}_{L_0} - {}^{k}_{L_0} \\ \hline {}^{b}_{L_1} - {}^{K}_{L_1}\end{matrix} \cdot \left(A^{1}_{L_1} - K_{L_1}\right)\right)}{\left(B_{L_0} - {}_{L_0}\right) - \left(\begin{matrix}{}^{a}_{L_0} - {}^{k}_{L_0} \\ \hline {}^{b}_{L_1} - {}^{K}_{L_1}\end{matrix} \cdot \left(B^{1}_{L_1} - K_{L_1}\right)\right)}$$

in which L_0 and L_1 represent the wave-lengths 380 mμ. and 385 mμ.; A, A^1, B and B^1 the extinctions at the wave-lengths L_0 and L_1 for the two samples in question; a and b the extinctions at L_0 and L_1 for the sample obtained immediately after the injection; and k and K the extinctions at the wave-lengths L_0 and L_1 for the sample obtained immediately before the injection.

The results are given graphically in Fig. 5.

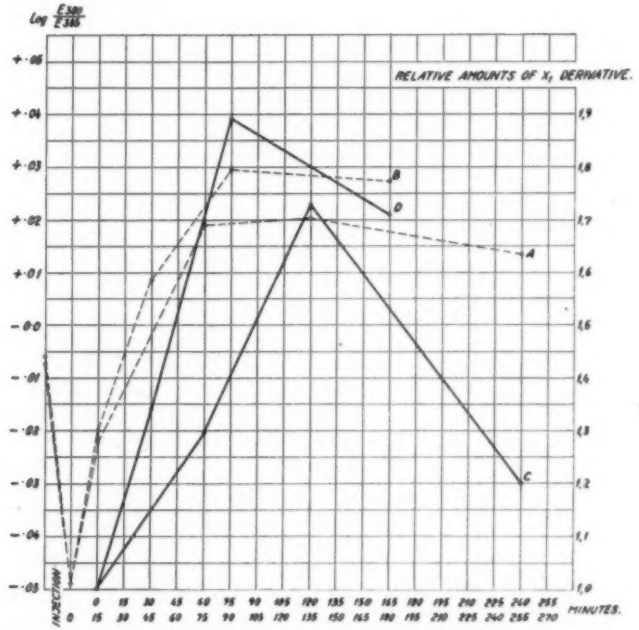

FIG. 5.—A and B show the variations in the quotient 380/385 mμ. (left ordinate axis). C and D give the corresponding relative amounts of X_2 derivative calculated in the way described (right ordinate axis).

From Fig. 5, curves C and D, and Table I, it is seen that both the conversion of 3:4-benzpyrene to the X_1 derivative and the elimination of the X_1 derivative from plasma (= conversion of X_1 to X_2 derivative ?) follow the equation for the rate of a monomolecular reaction.

SUMMARY.

In relation to an intravenous injection of 3:4-benzpyrene a decrease in the amount of nitrogen (protein) in plasma is observed. The decrease is of rather short duration, and is most conspicuous at the time when the conversion of 3:4-benzpyrene is most intense.

After intravenous injection of 3:4-benzpyrene in human beings only the absorption characteristic of the BPX_1 derivatives are observed.

TABLE I.

$$K = \frac{1}{t} \times \log \frac{a}{a-x}.$$

	t.	$\log \frac{a}{a-x}$.	K.
Fig. 5, curve D :			
	15	0·0434	0·002893
	30	0·0945	0·003150
	45	0·1496	0·003320
	60	0·2082	0·003470
	75	0·2764	0·003685
	15	0·0069	0·000460
	30	0·0141	0·000470
	75	0·0366	0·000488
Fig. 5, curve C :			
	30	0·0607	0·002023
	45	0·0899	0·001998
	60	0·1303	0·002171
	75	0·1553	0·002071
	90	0·1790	0·001989
	105	0·2122	0·002021
	120	0·2405	0·002004
	30	0·0390	0·00130
	45	0·0941	0·00125
	90	0·1614	0·00135

Experiments with rabbits indicate the probability that the conversion of 3:4-benzpyrene after intravenous injection takes place in the liver.

A method is given of estimating the process of conversion. Relative estimations of the amounts of X_1 derivative in plasma show that both the conversion of 3:4-benzpyrene to the X_1 derivative and the elimination of X_1 derivative from plasma follow the equation for the rate of a monomolecular reaction.

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